



# BLOOD

*The Journal of Hematology*

VOLUME II, 1947



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VOLUME 2, 1947

# BLOOD

## *The Journal of Hematology*

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## SPECIAL ISSUES 1 AND 2 CONTENTS

NOTE: *Simultaneously with the six regular issues of BLOOD 1947, two Special Issues were published: No. 1, Morphologic Hematology, July 1947; No. 2, The Rh Factor, January 1948 (originally scheduled for December 1947). As a convenience to readers, the contents of the Special Issues are given here. Likewise, Volume II index includes complete references to the Special Issues.*

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## *The Journal of Hematology*

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### A STATISTICAL STUDY OF MORTALITY FROM LEUKEMIA

By MILTON S. SACKS, M.D., AND ISADORE SEEMAN, B.S.

#### I. INTRODUCTION

**S**URVEY of the medical literature reveals a paucity of statistical data on leukemia morbidity and a virtual absence of mortality studies. Neilsen<sup>1</sup> in 1932 estimated the annual death rate from leukemia in Denmark at 1 per 50,000 population. Wintrobe<sup>2</sup> noted that according to statistics of the Metropolitan Life Insurance Company there were 3,500 deaths due to leukemia in the United States annually between 1934 and 1936. The application of biostatistical technic to the search for further knowledge of etiological factors in leukemia offers a threefold supplement to clinical and laboratory research. The results of careful statistical analysis of mortality from leukemia may serve to: (1) elucidate specifically the trends in mortality, (2) indicate needed areas for further study, and (3) demonstrate the numerical significance of the problem.

Two factors support the preferability of an analysis of mortality data: (1) compulsory registration of all deaths and the collection of statistics in a central authority provide almost complete reporting of deaths, and (2) experience indicates that the case fatality rate from leukemia is 100 per cent, insuring the reporting on a death certificate of almost every diagnosed case. In spite of the advantages of analyzing the incidence of leukemia through mortality figures, a number of cautions must be observed in interpreting death statistics in general.

1. Changes in the age distribution of a population will introduce a spurious element into crude death rate trends over a period of years, particularly for diseases influenced largely by the factor of age.

2. Death statistics are derived from physicians' statements of cause of death on death certificates. The care exercised in the execution of a medical certification is reflected in mortality statistics.

3. Improvement in diagnostic technics for any disease will tend to increase the reporting of the incidence of that disease on case and death records.

4. For the United States, mortality data prior to 1933 represent figures from an incomplete but expanding geographical area.

5. Changes in the procedures of mortality tabulation introduce an element of discontinuity in published mortality statistics. Such changes result from revisions of the International List of Causes of Death, revisions of the Manual of

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Joint Causes of Death used in the United States to select one cause for statistical tabulation when several causes are certified jointly, and other procedural revisions.

Each of these factors is considered in the present study, the purpose of which is to review the trends of mortality from leukemia from 1900 to 1944.

*Influence of Procedural Changes on Leukemia: Mortality Statistics*

Since 1900 there have been five revisions of the International List of Causes of Death. In each revision a rubric for leukemia has been provided. Until the revision of 1920 only a single title under which Hodgkin's disease was also classified was assigned for leukemia. It is impossible, therefore, to discuss mortality from leukemia from 1900 to 1920 without recognizing the exaggeration inherent in the figures as a result of the inclusion of more or less closely allied conditions.

With the revision of 1920 a subclassification for pseudoleukemia was established to make possible the classification of Hodgkin's disease separately from true leukemia. Relatively minor changes in specific terms under the rubric for true leukemia have been made since 1920. In the discussion of leukemia mortality in the United States since 1920, only the figures for true leukemia have been used in this paper.

It must be noted, however, that the omission of all deaths charged to pseudoleukemia from 1920 to 1938 results in an understatement of the mortality from the leukemic process. This fact was demonstrated in 1940 when a comparative analysis of deaths in that year classified under both the revision of 1929 and the revision of 1938 was made by the U. S. Bureau of the Census.<sup>3</sup> This study revealed that in 1940, 2,139 deaths would have been charged to the pseudoleukemia title of the 1929 list. Of these deaths, 1,777 or 83 per cent were due to Hodgkin's disease and 247 or 11 per cent were due to aleukemia. Aleukemia, in reality a form of leukemia, appears to constitute a significant proportion of the deaths attributed to the pseudoleukemia title. Since the entire rubric of pseudoleukemia has been excluded from this study, the figures from 1920 to 1938 obviously err on the conservative side.

In the revision of 1938 currently in use,<sup>4</sup> the rubric for pseudoleukemia was discontinued, and diseases so certified were classified under nonspecific diseases of the blood; a separate classification for Hodgkin's disease was provided in the residual group of communicable diseases; and a new subrubric for aleukemias was established. To maintain continuity, only the leukemia figures since 1938 have been used in this study.

All of these changes in the categories assigned to leukemias and allied disorders in the International List reflect changing medical concepts concerning these diseases. As further data on their etiology accumulate, revisions in their classification may be expected.\* It is apparent, then, that problems of classification added to those of diagnosis and reporting result in an understatement of the number of deaths from leukemia.

\*Preliminary consideration of the forthcoming revision of the International List of Causes of Deaths has tentatively assigned the leukemia title in the major group assigned to tumors.

Changes in the rules for joint-cause selection have had no significant effect on the trend of recorded mortality from leukemia.

## II. UNITED STATES EXPERIENCE IN LEUKEMIA MORTALITY

The crude mortality rate from leukemia in the United States Death Registration States has risen from 1.0 per 100,000 population in 1900 to 4.3 per 100,000 in 1944, an increase of 330 per cent<sup>5-8</sup> (fig. 1 and table 1). The apparent drop in the death rate in 1921 observed in figure 1 results primarily from the revision of this title in 1920.

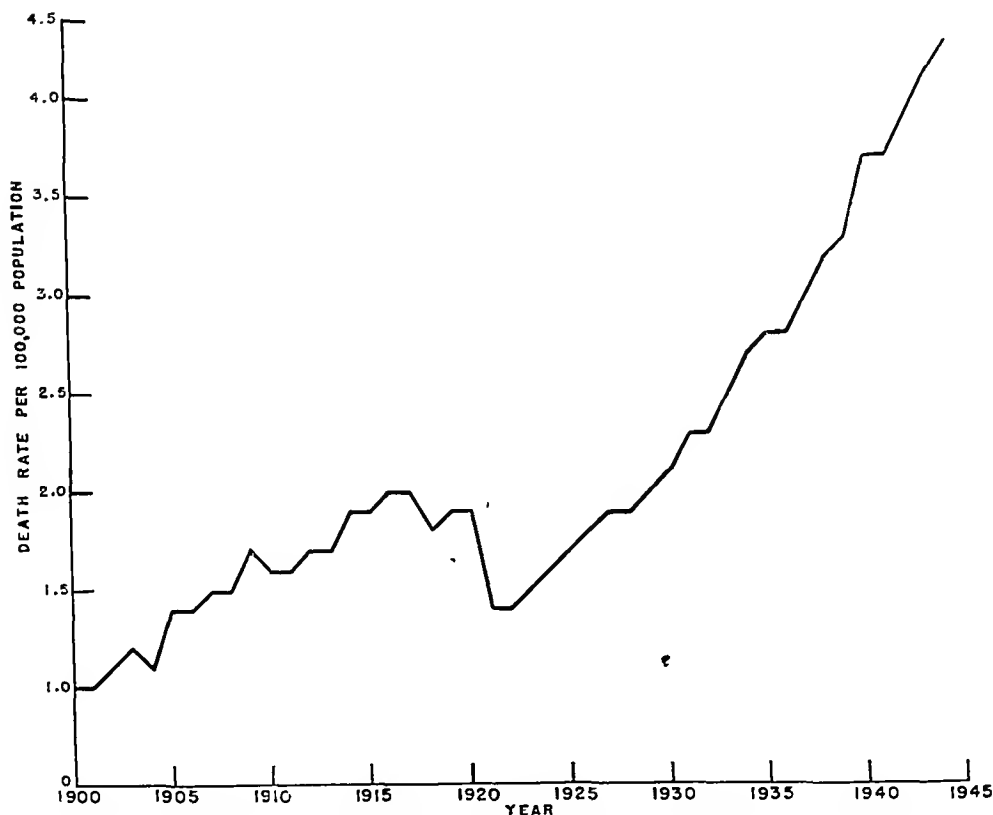


FIG. 1. CRUDE DEATH RATE FROM LEUKEMIA, UNITED STATES DEATH REGISTRATION STATES, 1900-1944

### *Influences of Race, Sex, and Age*

The data (table 2) indicate that leukemia affects the white population in the United States at a rate nearly two and one-half times as great as that for nonwhites. Thus, the average death rates per 100,000 population for the Death Registration States from 1925 through 1940 were 2.7 for white persons and 1.1 for colored persons (fig. 2). The causes for this consistent difference are not entirely obvious

at this time. Undoubtedly some of this difference is accounted for by differences in the availability of thorough medical diagnosis for these two groups.

Males are affected by leukemia at a rate one-third greater than that for females.

TABLE 1.—*Mortality from Leukemia, by Race and Sex, United States Death Registration States, 1921-1942*

Year	Death Rate per 100,000 Population	Number of deaths						
		Total	White			Nonwhite		
			Total	Male	Female	Total	Male	Female
Leukemias (74a)*								
1942	3.9	5,189	4,947	2,838	2,109	242	145	97
1941	3.7	4,954	4,705	2,715	1,990	249	143	106
1940	3.7	4,896	4,646	2,719	1,927	250	160	90
1939	3.3	4,280	4,077	2,336	1,741	203	113	90
True Leukemias (72a)†								
1938	3.2	4,126	3,922	2,287	1,635	204	116	88
1937	3.0	3,899	3,702	2,115	1,587	197	109	88
1936	2.8	3,628	3,476	2,004	1,472	152	82	70
1935	2.8	3,552	3,391	1,953	1,438	161	100	61
1934	2.7	3,403	3,227	1,805	1,422	176	95	81
1933	2.5	3,088	2,959	1,725	1,234	129	84	45
1932	2.3	2,770	2,669	1,509	1,160	101	63	38
1931	2.3	2,692	2,583	1,511	1,072	109	64	45
1930	2.1	2,508	2,400	1,412	988	108	73	35
Leukemia (65a)‡								
1929	2.0	2,259	2,174	1,238	936	85	49	36
1928	1.9	2,185	2,097	1,180	917	88	50	38
1927	1.9	1,997	1,931	1,098	833	66	35	31
1926	1.8	1,854	1,786	1,013	773	68	37	31
1925	1.7	1,759	1,689	999	690	70	41	29
1924	1.6	1,543	1,481	869	612	62	40	22
1923	1.5	1,424	1,366	774	592	58	36	22
1922	1.4	1,343	1,290	748	542	53	30	23
1921	1.4	1,217	1,179	676	503	38	18	20

\* International List of Causes of Death, Fifth Revision, 1938.

† International List of Causes of Death, Fourth Revision, 1929.

‡ International List of Causes of Death, Third Revision, 1920.

The average death rates per 100,000 males from 1925 through 1940 was 2.9; the corresponding rate for females was 2.2 per 100,000.

The death rate for all groups is highest in the ages after 55 years, with the peak, a rate of 15.7 deaths per 100,000 population, occurring between 75 and 84 years, and remaining high at even older ages. A secondary peak at ages under 5 years is

noted. The rates are distinctly lower in the years 5 through 44, after which a definite rise occurs.

An analysis of the death rates from leukemia in the United States for the years 1931 and 1940, specific for age, race, and sex, affords some interesting observations (table 3, fig. 3). The rates for the two years show the same general relationships. In 1940 the death rate for white males is exceeded by no other group at any age except over 85 years, where colored males have a slightly higher rate. The rate for white males reaches a maximum of 21.1 deaths per 100,000 population in the age group 75-84 years. The rate for colored males is greater than the rate for colored females at all ages after the first year; it exceeds that for white females between

TABLE 2.—*Leukemia Death Rate per 100,000 Population, by Race and Sex, United States Death Registration States, 1925-1940*

Year	Total				White		Nonwhite	
	White	Nonwhite	Male	Female	Male	Female	Male	Female
1940	3.9	1.9	4.3	3.1	4.6	3.3	2.4	1.3
1939	3.5	1.5	3.7	2.8	3.9	3.0	1.7	1.3
1938	3.4	1.5	3.7	2.7	3.9	2.8	1.8	1.3
1937	3.2	1.5	3.4	2.6	3.6	2.8	1.7	1.3
1936	3.0	1.2	3.2	2.4	3.5	2.6	1.3	1.1
1935	3.0	1.2	3.2	2.4	3.4	2.5	1.5	0.9
1934	2.8	1.4	3.0	2.4	3.1	2.5	1.5	1.2
1933	2.6	1.0	2.9	2.1	3.0	2.2	1.3	0.7
1932	2.5	0.9	2.6	2.0	2.8	2.2	1.1	0.6
1931	2.4	0.9	2.6	1.9	2.8	2.0	1.1	0.8
1930	2.3	0.9	2.5	1.8	2.6	1.9	1.3	0.6
1929	2.1	0.7	2.2	1.7	2.3	1.8	0.9	0.6
1928	2.1	0.8	2.1	1.7	2.3	1.8	0.9	0.7
1927	2.0	0.7	2.1	1.6	2.2	1.7	0.7	0.6
1926	1.9	0.7	2.0	1.6	2.1	1.7	0.8	0.7
1925	1.8	0.8	2.0	1.4	2.1	1.5	0.9	0.6

15 and 34 years. The rate for white females exceeds that for colored females at all ages.

When the total death rate from leukemia from 1921 to 1940 is adjusted for age and sex to a standard population, the trend in the adjusted rate is seen to follow the crude death rate trend very closely (table 4). The percentage increase in the adjusted rate from 1921 to 1925 and from 1925 to 1940 is exactly the same as the percentage increase in the crude rate. From 1930 to 1935 and from 1935 to 1940 the percentage increase in the adjusted rate is slightly below that for the crude rate. It is thus apparent that the rise in the total death rate from leukemia from 1921 to 1940 is due in only very small part to a changing age distribution of the population.

Review of the change in the death rate from leukemia in each age group from 1931 to 1940 indicates that the greatest increases are occurring at those ages where

the rate is highest. Thus, while the death rate at all ages increased by 61 per cent from 1931 to 1940, the average percentage increase for all ages over 64 was 104 per cent (table 5).

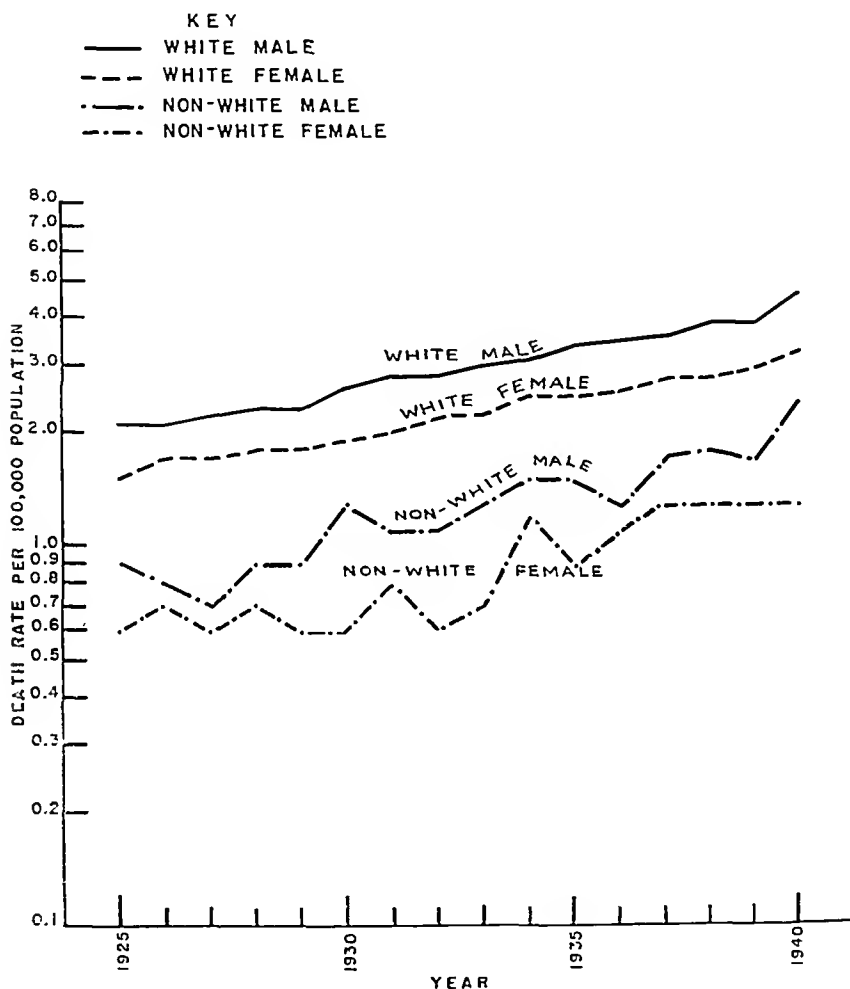


FIG. 2. DEATH RATE FROM LEUKEMIA BY RACE AND SEX, UNITED STATES  
DEATH REGISTRATION STATES, 1925-1940  
(logarithmic scale)

It becomes obvious, therefore, that under present conditions a continued rise in the total death rate from leukemia is to be anticipated, since the number and percentage of the population in the older ages most affected by leukemia continues to increase, and at the same time these age groups are affected by leukemia at an increasing rate.

TABLE 3.—*Leukemia Death Rates per 100,000 Population, Specific for Age, Race, and Sex, United States Death Registration States, 1931 and 1940*

Race and Sex	All Ages	Under 1 Year	1-4 Years	5-14 Years	15-24 Years	25-34 Years	35-44 Years	45-54 Years	55-64 Years	65-74 Years	75-84 Years	85 Years and Over
1940												
Total.....	3.7	4.9	4.5	1.9	1.5	1.6	2.6	4.9	8.7	11.9	15.7	6.9
White.....	3.9	5.2	4.9	2.0	1.5	1.6	2.7	5.0	8.9	12.6	16.3	7.1
Male.....	4.6	5.6	5.6	2.4	1.9	1.7	3.1	5.5	10.5	14.9	21.1	6.3
Female....	3.3	4.8	4.1	1.6	1.1	1.5	2.3	4.5	7.3	10.4	11.9	7.6
Nonwhite . . . . .	1.9	2.5	2.0	0.7	1.1	1.2	2.2	3.6	5.1	3.1	4.7	5.5
Male.....	2.4	2.5	2.4	1.0	1.4	1.7	2.3	4.2	6.5	5.3	8.1	6.4
Female....	1.3	2.5	1.5	0.4	0.7	0.8	2.1	3.0	3.5	0.9	1.5	4.8
1931												
Total.....	2.3	2.8	2.9	1.3	1.0	1.2	1.9	3.2	5.3	7.5	5.9	3.7
White.....	2.4	2.9	3.2	1.4	1.1	1.3	2.0	3.4	5.6	7.9	6.1	4.1
Male.....	2.8	2.8	3.6	1.7	1.4	1.4	2.2	3.6	6.5	9.2	7.9	6.7
Female....	2.0	3.1	2.8	1.1	0.8	1.1	1.8	3.1	4.6	6.5	4.4	2.1
Nonwhite.....	0.9	1.8	0.4	0.7	0.8	0.9	1.1	1.3	1.4	1.1	3.2	—
Male.....	1.1	1.8	0.6	1.2	1.1	0.7	1.4	1.5	0.6	1.4	2.2	—
Female....	0.8	1.7	0.2	0.2	0.5	1.1	0.7	1.1	2.5	0.8	4.2	—

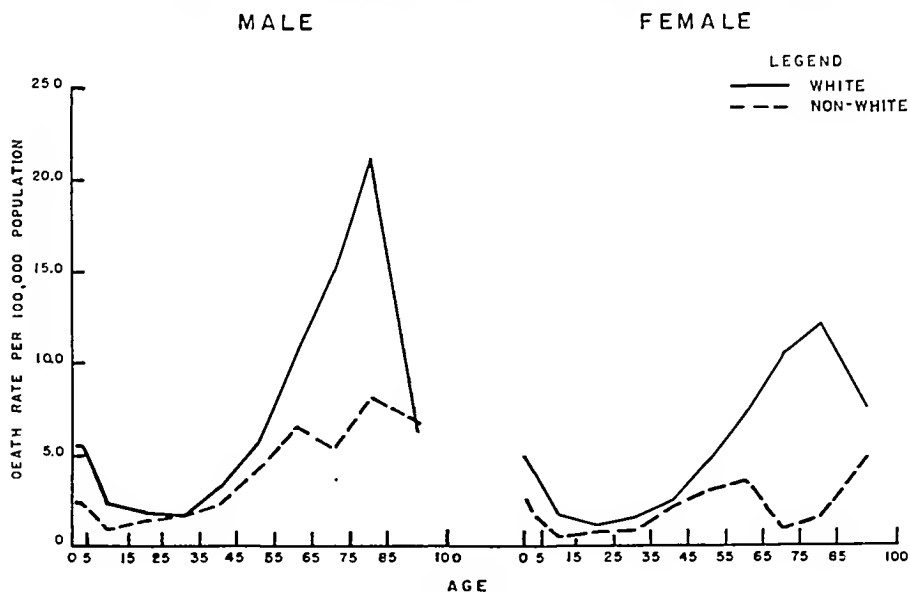


FIG. 3. DEATH RATES FROM LEUKEMIA SPECIFIC FOR AGE, RACE, AND SEX, UNITED STATES, 1940

*Factors of Cell Type and Chronicity*

Data on cell type and chronicity are not available for the United States as a whole. Special analysis was therefore made of the original records of 154 deaths from leukemia in Baltimore filed with the Baltimore City Health Department during the five year period 1939 through 1943. It was observed that the deaths with cell type specified, constituting 87 per cent of the total deaths, indicated an almost equal incidence of lymphoid and myeloid leukemias. Only 63 per cent of the records indicated chronicity; of these deaths 61 per cent were acute and 39

TABLE 4.—*Trend in Crude and Age-Sex Adjusted Death Rates from Leukemia, United States Death Registration States, for Selected Years, 1921-1940*

Year	Crude Death Rate per 100,000 Population	Age-Sex Adjusted Death Rate per 100,000 Population*	Percentage Increase Over Previous Date	
			Crude Rate	Adjusted Rate
1921	1.4	1.4		
1925	1.7	1.7	21.4	21.4
1930	2.1	2.1	23.5	23.5
1935	2.8	2.7	33.3	28.6
1940	3.7	3.5	32.1	29.6

\* Adjusted to Standard Million Population from U. S. Census of 1930.

TABLE 5.—*Percentage Increase in Age-Specific Leukemia Death Rates, 1931-1940, United States Death Registration States*

Age Group	Percentage Increase 1931 to 1940
All ages	60.9
Under 1 year	75.0
1-4 years	55.2
5-14 years	46.1
15-24 years	50.0
25-34 years	33.3
35-44 years	36.8
45-54 years	53.1
55-64 years	64.1
65-74 years	58.7
75-84 years	166.1
85 years and over	86.5

per cent chronic. Of the deaths with cell type and chronicity specified, 60 per cent of the myeloid cases and 55 per cent of the lymphoid cases were acute.

As has been noted in other studies,<sup>9</sup> the occurrence of acute leukemia is characteristic of younger ages while chronic leukemia affects the older segments of the population. In the Baltimore experience studied (table 6), acute leukemia occurred most commonly in children under 5 years of age. In general leukemia of an acute character affected persons from 5 through 44 years more frequently than chronic leukemia. After age 45 the death rate from chronic leukemia rises markedly.

There appears to be no very significant age selection in the case of leukemias of lymphoid or myeloid cell type.

Race and sex appear to affect the incidence of acute and chronic leukemias of different cell type only insignificantly.

### *Causes of Death Associated with Leukemia*

The Manual of Joint Causes of Death<sup>4</sup> used by the United States Bureau of the Census gives preference to a large number of conditions when certified jointly

TABLE 6.—Average Annual Death Rate from Leukemia per 100,000 Population, by Chronicity, Cell Origin, and Age, Baltimore, 1939-1943

Chronicity and Cell Origin	Age										
	All Ages	-1	1-4	5-14	15-24	25-34	35-44	45-54	55-64	65-74	75+
Total .....	3.59	1.96	5.66	2.05	2.31	1.70	2.66	3.69	7.55	12.06	10.25
Acute .....	1.37	1.96	3.92	1.11	1.16	1.05	1.33	.74	1.74	1.51	3.84
Lymphoid .....	.58	—	2.18	.79	.51	.52	.29	.18	.87	.50	—
Myeloid .....	.61	—	.87	.16	.64	.39	.89	.37	.87	1.00	2.56
Monocytic .....	.07	—	—	—	—	—	.15	.18	—	—	1.28
Unspecified .....	.12	1.96	.87	.16	—	.13	—	—	—	—	—
Chronic .....	.88	—	—	.31	.13	.26	.74	1.48	3.19	3.52	2.56
Lymphoid .....	.47	—	—	.31	—	—	.15	.74	1.74	3.01	1.28
Myeloid .....	.40	—	—	—	.13	.26	.59	.74	1.45	.50	—
Monocytic .....	—	—	—	—	—	—	—	—	—	—	—
Unspecified .....	.02	—	—	—	—	—	—	—	—	—	1.28
Unspecified .....	1.33	—	1.74	.63	1.03	.39	.59	1.48	2.61	7.03	3.84
Lymphoid .....	.49	—	1.31	.47	.26	—	.29	.74	.58	1.51	2.56
Myeloid .....	.49	—	—	.16	.51	.26	.15	.55	1.16	3.01	—
Monocytic .....	.02	—	—	—	—	—	—	—	.29	—	—
Unspecified .....	.33	—	.43	—	.26	.13	.15	.18	.58	2.51	1.28
Lymphoid .....	1.54	—	3.48	1.58	0.77	0.52	0.74	1.66	3.19	5.02	0.38
Myeloid .....	1.49	—	0.87	0.31	1.28	0.92	1.62	1.66	3.48	4.52	0.25

with leukemia in assignment as the underlying cause of death. Chief among these are the acute communicable diseases, tuberculosis, the venereal diseases, cancer, maternal deaths, and deaths from violence or accidents. Joint-cause studies for the United States have been made for the years 1917, 1925, and 1940 in an effort to reveal the relationship between underlying causes of death and associated conditions. In all three of these years more than 95 per cent of all certificates containing leukemia were classified to this cause by Census-Bureau procedures.

In 1940, 2,381 death certificates listed other causes in addition to leukemia.

Analysis of these records reveals possible sources of error in diagnosis, reporting, or tabulation procedure which might result in understatement of the true mortality from leukemia. There were 195 death certificates assigned to some cause other than leukemia stated on the certificate. In 72 of these cases the death was assigned to cancer, although in a majority of instances the nature or site of the neoplasm was unknown. Of the remaining 2,186 joint-cause certificates which were assigned to leukemia, 15 per cent listed anemia or a disease of the spleen associated with leukemia. There were 241 records listing both leukemia and anemia. During the year 1940, United States figures reveal that the cause of death on 973 certificates was assigned to anemias other than pernicious anemia. Thus for every 4 deaths charged to anemia, there was 1 death in which the anemia was recognized as an associated condition in the leukemic process. Ninety-one death certificates listed both leukemia and a disease of the spleen. During this same year 508 deaths were charged to splenic disease. Thus for every 6 deaths assigned to splenic disease there was 1 in which the splenic disease was recognized as an associated factor in

TABLE 7.—*Death Rate from Leukemia in Census Tracts Grouped According to Median Monthly Rent, Baltimore 1939-1943*

Median Contract or Estimated Monthly Rent	Population Census April 1, 1940	Total Leukemia Deaths 1939-1943	Death Rate per 100,000 Population	Leukemia Deaths in Hospitals	Per cent of Leukemia Deaths in Hospitals
Total	853,578	154	3.6	115	74.7
Under \$15.00	61,221	10	3.3	8	80.0
\$15-19.99	85,374	17	4.0	13	76.5
20-24.99	241,219	34	2.8	29	85.3
25-29.99	167,582	24	2.9	18	75.0
30-34.99	121,410	21	3.5	17	80.9
34-39.99	56,612	10	3.5	8	80.0
40-44.99	53,766	17	6.3	10	58.8
45 and over	66,394	25	7.5	12	48.0

the leukemic process. These two categories of disease must therefore be recognized as potential sources of additional case material for statistical study.

#### *Influence of the Economic Factor*

By the use of available data on monthly rental in each of the 157 small geographic areas in Baltimore designated as census tracts,<sup>10</sup> it is possible to divide the city into seven segments ranging in economic status from those with a median rental of less than \$15 per month to those with a median monthly rent of \$45 or more. Analysis of the death rate from leukemia in each of these economic segments reveals a rising death rate paralleling a rise in economic status in each of these segments except for the two lowest economic groups, where the rate is higher than in the intermediate groups (table 7). Perhaps this trend may be accounted for in part by the greater access to more exhaustive diagnostic facilities for families with larger personal financial resources and those with the poorest resources to whom medical aid at public expense is available.

Analysis of the deaths in Baltimore in the five year period 1939-1943 indicates that 74.7 per cent of the deaths from leukemia occurred in hospitals (table 7). This compares with a mean percentage of hospital deaths from all causes of 41.1 for the same period. Although the economic segments with the highest death rates have the lowest percentage of hospital deaths from leukemia, in many instances the diagnosis was undoubtedly made during a period of hospitalization after which the patients in this group returned home before death to secure the comforts which their financial status made possible.

### III. INTERNATIONAL EXPERIENCE IN LEUKEMIA MORTALITY

Comparison of the death rate from leukemia and pseudoleukemia\* in the year 1931 for England and Wales,<sup>11</sup> Canada, the city of Paris<sup>12</sup> and the United States

TABLE 8.—*Death Rates per 100,000 Population from Leukemia and Pseudoleukemia, United States, England and Wales, Canada, and Paris, 1931*

Age	United States*			England and Wales			Paris			Canada†		
	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female
A: Age-Sex Adjusted Death Rates‡												
All ages	3.48	4.09	2.85	3.03	3.75	2.29	2.53	3.22	1.83	2.29	2.75	1.82
B. Age-Sex Specific Death Rates												
Under 5		3.71	2.97		3.03	2.35		1.34	—		1.84	1.88
5-14		2.16	1.33		2.69	1.48		3.81	2.26		1.34	0.73
15-24		2.18	1.32		2.36	1.31		2.72	0.81		1.41	1.35
24-44		3.33	2.35		3.35	2.03		2.89	1.49		2.02	1.56
45-64		7.42	5.43		5.83	3.98		4.12	3.27		5.19	3.16
65 and over		11.43	8.18		8.92	4.71		4.76	4.30		9.86	4.62

\* White population only for the U. S. Death Registration States.

† Exclusive of Yukon and the Northwest Territories.

‡ Death rates at all ages have been adjusted for age and sex to the Standard Million Population of the U. S. Census of 1930.

Death Registration States indicates that the general experience corresponds closely with that observed for the United States. For greater comparability, only the white population of the United States has been included (table 8).

To arrive at a total death rate for each community which is comparable in spite of differences in population with respect to age and sex composition, death rates adjusted for age and sex have been computed. The age-sex adjusted death rates per 100,000 population for 1931 were: United States, 3.5; England and Wales, 3.0; Paris, 2.5; Canada, 2.3. When it is remembered that procedural variations in cause-of-death classification exercise an influence in international comparability

\*Only the combined figures for leukemia and pseudoleukemia were available for some of the communities studied.

of mortality statistics, the small differences in these observed rates indicate a very similar experience in each of the communities studied.

The incidence of leukemia in each sex as revealed by the age adjusted death rates for each area shows some variations. In the United States the death rate for males was 43 per cent greater than for females. The difference in rates by sex for Canada was 51 per cent, for England and Wales, 64 per cent, and for Paris, 76 per cent, with the rate for males higher in each case.

Analysis of the age distribution of the deaths in each area substantiates the observations for the United States, with the highest age specific rates occurring in the groups 45 years and older and a secondary high in the ages under 5. Some deviations from the general trend are observed: (1) the death rates in Canada in the age group 65 years and over are relatively higher than in the other areas, and (2) the ages under 5 experience the lowest rate in Paris, with the secondary peak occurring in the group 5-14 years.

TABLE 9.—*Death Rates per 100,000 Population for Selected Causes, United States Death Registration States by Five Year Periods, 1920-1940*

Cause of Death	Death Rate per 100,000 Population				
	1940	1935	1930	1925	1920
Leukemia . . . . .	3.7	2.8	2.1	1.7	1.9
Exophthalmic goiter . . . . .	2.8	2.8	3.4	3.4	1.8
Whooping cough . . . . .	2.2	3.7	4.8	6.7	12.5
Dysentery . . . . .	1.9	1.9	2.8	3.1	4.0
Alcoholism . . . . .	1.9	2.6	3.5	3.6	1.0
Diphtheria . . . . .	1.1	3.1	4.9	7.8	15.3
Malaria . . . . .	1.1	3.5	2.9	2.0	3.4
Typhoid & paratyphoid fever . . . . .	1.1	2.8	4.8	7.8	7.6
Poliomyelitis . . . . .	0.8	0.8	1.2	1.5	0.9
Scarlet fever . . . . .	0.5	2.1	1.9	2.7	4.6
Meningococcus meningitis . . . . .	0.5	2.1	3.6	1.0	1.6
Smallpox . . . . .	0.0	0.0	0.1	0.7	0.6

#### IV. THE SIGNIFICANCE OF MORTALITY FROM LEUKEMIA

In 1942 more persons in the United States died from the leukemias than from smallpox, meningococcus meningitis, scarlet fever, poliomyelitis, malaria, typhoid fever, and diphtheria combined.<sup>7</sup> The death rate from leukemia was higher than that for the anemias, whooping cough, the dysenteries, or alcoholism. Each year since 1940 more than 5,000 persons have died of the leukemias in the United States.

The leukemia death rate in the U. S. Death Registration States rose from 1.9 per 100,000 population in 1920 to 3.7 in 1940, an increase of 94.7 per cent (table 9). The death rate from typhoid fever during the same twenty-year period fell from 7.6 per 100,000 population to 1.0, a decline of 86.8 per cent. Decreases of 98.4 per cent for diphtheria, 89.1 per cent for scarlet fever, and 82.4 per cent for whooping cough were experienced during this period. While much of the apparent rise in the leukemia rate is undoubtedly artificial and attributable to better diagnosis and more complete reporting, it must still be observed that leukemia has been a

more important cause of death during the last twenty years than many of those diseases generally recognized as health hazards. The latter group which have traditionally received the attention of official departments of health are, of course, in the main of a communicable nature. The neglect of those more widespread causes of death whose etiology and control are largely beyond present knowledge is a common failure of public health administration and finance. That this failure applies to the field of research as well is shown in an analysis of funds contributed for this purpose by foundations in 1940. Data from a study by Medical Memorial Funds, Inc.,<sup>13</sup> show that in 1940 there was spent in research on poliomyelitis an average of \$502.00 per death. For research in all other infectious diseases an average of \$4.00 was contributed for each death. Corresponding figures for cancer were \$2.19 per death; diseases of the kidneys, \$0.38 per death; and diseases of the heart, \$0.17 per death. It is significant to note that leukemia was entirely absent from the research studies listed in this report for 1940. Such funds as may now be available for research in leukemia are almost negligible.

#### SUMMARY

The recorded death rate from leukemia in the United States has risen continuously since 1900, with an accelerated rate of increase since 1930. The rise from a rate of 1.9 per 100,000 population in 1920 to 3.7 in 1940 represents an increase of 94.7 per cent in this twenty-year period. This increase cannot be accounted for by changes in the age distribution of the population, for the age specific death rates have increased in each age group. The factor of increasing recognition of the disease resulting from improved diagnostic technics and greater use of hospitals with their laboratory facilities must be given adequate consideration in an effort to determine the causes for the rising death rate.

White persons are affected at a rate more than twice as great as nonwhites. Some of the difference must be attributed to variations in the availability of diagnostic services. Males experience a rate approximately one-third greater than females.

Leukemia affects persons in the older ages, particularly over 55 years, with the greatest frequency, and the population under 5 years of age experiences a mortality rate higher than any other age under 45 years. In the intermediate ages the death rate falls to the lowest point. In 1940 the death rate from leukemia for all ages was 3.7 per 100,000 population. The highest rate, 15.7 per 100,000 occurred in the age group 75-84 years. Under 1 year the rate was 4.9 per 100,000. The lowest rate, 1.5 per 100,000, occurred in the ages from 15 to 24 years.

Figures for the city of Baltimore for the five-year period 1939-1943 indicate an almost equal incidence of lymphoid and myeloid leukemia. Nearly two-thirds of the deaths studied in Baltimore were reported as acute leukemia. Acute myeloid leukemia appears to be more common than acute lymphoid. After age 45 chronic leukemia is more frequently observed; younger persons experience acute leukemia most commonly.

Undoubtedly many deaths result from leukemia in which this disease was neither diagnosed nor recorded on a death certificate. Clinical evidence indicates

that the causes in which this failure would occur most commonly are cancer, anemia, and diseases of the spleen. Statistical evidence reveals that these conditions are certified jointly with leukemia in a significant number and proportion of cases.

Comparison of the experience of several countries indicates that the general trends of mortality from leukemia in the United States are common to the other communities. The death rates per 100,000 population in 1931 adjusted for differences in age and sex composition of the population were: United States, 3.5; England and Wales, 3.0; Paris 2.5; and Canada 2.3.

Each year since 1940 more than 5,000 persons in the United States have died from leukemia.

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## THE TREATMENT OF LYMPHOBLASTIC LEUKEMIA WITH CRUDE MYELOKENTRIC ACID

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EVIDENCE has been presented previously that myelokentric acid and lymphokentric acid<sup>1</sup> are present in varying amounts in the urine of patients with acute and chronic leukemias, Hodgkin's disease, and lymphosarcoma. We reported in 1940<sup>2</sup> that partial remissions occurred in 2 patients with lymphoblastic leukemia during periods in which they were receiving injections of extracts of the urine from patients with chronic myeloid leukemia. At that time it was postulated that patients with lymphoblastic leukemia lacked the stimulus for myeloid proliferation. Later evidence was given that substantiates the hypothesis of a balance between myeloid and lymphoid cell production.<sup>3</sup>

The present paper reports 12 cases of lymphoblastic leukemia. To 8 of them we have given extracts of urine or feces from patients with chronic myeloid leukemia. The other 4 cases are reported as controls. Necropsies were performed on 5 of the 8 treated cases and on all 4 untreated cases. A comparison is drawn between the clinical course and pathologic morphology of treated and untreated cases.

No patients were especially selected either for treatment or for use as controls. All were relatively young individuals, the oldest being 30 years of age in the untreated group. The treated group ranged from 2½ years of age to 15 years of age.

All patients, treated or controls, were given blood transfusions when necessary. Bone marrow aspirations were done in all instances in order to ascertain the extent of the disease. In some of the treated cases bone marrow aspirations were repeated when partial remission occurred. Four of the patients were treated with either sulfadiazine or penicillin when infection occurred.

The crude myelokentric acid that was used was extracted either from urine or feces of patients with chronic myeloid leukemia. The extracts were made by methods we have described. Chloroform extract of hydrolyzed urine was used in the treatment of 5 cases.<sup>4</sup> Chloroform extract of feces was given to 1 patient,<sup>1</sup> and to 2 patients the hydrolyzed eluate of kaolin adsorbate of urine was given.<sup>5</sup> When the latter was used the dose was increased ten to fifteen fold because of the difference in potency of the material as determined by assay on guinea pigs.<sup>6</sup>

### CASE REPORTS

*Case 1.* J. H., a 5 year old white girl, had whooping cough in November 1938 and shortly afterwards it was noticed that she was pale. From January 1939 to October 1939 she received four blood transfusions. She entered the Jefferson Hospital in October 1939 because of a persistently low leukocyte count. A sternal marrow aspiration and a biopsy revealed marrow made up largely of lymphoblasts. She was given several transfusions, liver extract, and vitamins. The leukocyte count varied from 850 to 4,500. The spleen and liver were both palpable at all times and there was some enlargement of all peripheral lymph nodes. Because it was thought that she might have splenic neutropenia, a splenectomy was done 12-4-39. The spleen weighed 130 grams and two accessory spleens were found and removed. The splenic pulp consisted largely of lymphoblasts.

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During the four weeks following splenectomy a mild remission occurred, and normal blood cell elements appeared in the peripheral blood. A bone marrow aspiration three weeks after splenectomy revealed nearly normal hematopoiesis. On 1-11-40 relapse was evident. At this time the leukocyte count had dropped from 7,000 to 2,000 and lymphoblasts were occasionally found in the peripheral blood. On January 13 she was given the first dose of crude myelokentric acid. This was the chloroform extract of hydrolyzed urine, and it was given in alkaline water at pH 7.5 in doses of 2 or 3 cc. every other day; each cc. represented 260 cc. of urine. With each injection of myelokentric acid 1 to 3 units of liver extract were given. At the end of two weeks the leukocyte count was being maintained at or above 4,500 per cu. mm., with 30 per cent polymorphonuclear neutrophils. At this time she was given no extract for seven days and the leukocyte count fell to 2,000 per cu. mm. Treatment with the extract was resumed in doses five days apart till 2-15-40, when daily doses of 1 to 1½ cc. were given for the following four weeks. Throughout this time she appeared to be well into remission. The bone marrow had again approached normal in cellular elements and the leukocyte count and erythrocyte count were well maintained. On 3-11-40 active material was stopped and the inactive neutral fraction of urine extract was given instead. Injections of this were given in oil and she received an amount equal to 500 or 750 cc. of urine daily for six weeks. By the end of the fifth week of this treatment lymphoblasts again appeared in the blood stream, lymphocytes increased in percentage, and the polymorphonuclear neutrophils decreased. On 4-25-40 active material was again started and was continued for four weeks, but during this period she continued to go deeper into relapse. The leukocyte count remained high, 20,000 per cu. mm., and 40 per cent of the cells were lymphoblasts. The last sixteen days of her life she was again given the inactive neutral fraction of urine extract. The leukocyte count reached 60,000 but dropped to 1,200 the day before she died. Death occurred 6-9-40 and a necropsy was performed.

*Case 2.* S. G., a 15 year old white boy, entered Jefferson Hospital 1-15-40 because of pallor and pains in his legs and back. He had become ill early in November 1939. In December of that year he was admitted to a hospital where anemia was found and one transfusion was given. On entry to Jefferson Hospital the liver and spleen and all peripheral lymph nodes were moderately enlarged. The leukocyte count was 12,000 per cu. mm., 80 per cent of which were lymphoblasts. The erythrocyte count was 1,200,000 and hemoglobin 28 per cent. A bone marrow aspiration revealed a very cellular marrow made up almost entirely of lymphoblasts (fig. 1). The spleen, liver, and thoracic lymph nodes were enlarged clinically and were determined to be so by roentgenogram. On 1-19-40 an initial dose of crude myelokentric acid was given and this was followed by daily doses of 2 cc. (1 cc. equaling 400 cc. of urine). It was administered in alkaline water solution at a pH of 7.5. This was continued, with but few exceptions, daily until 3-8-40. One to 3 units of liver extract were given with nearly every injection of the urinary extract. During the first four weeks of this period he had many furuncles and abscesses on his arms and legs and his temperature varied from 100° to 104° F. Blood transfusions were given when necessary. In the first three weeks of this period three blood cultures were reported to show no growth in forty-eight hours, and agglutination tests for typhoid organisms were negative.

The leukocyte count fell from 12,000 to 300 during the first four weeks of treatment and the lymphoblasts decreased in percentages as well as in number, while mature lymphocytes appeared in the low counts. After the marked leukopenia of 300 was reached, the leukocyte count slowly began to rise and as it rose normal leukocytes of both the myeloid and the lymphoid series made up the blood picture. On 3-8-40 the leukocyte count was 3,400 and the differential count was 36 per cent neutrophils, 35 per cent metamyelocytes, 27 per cent normal lymphocytes, and 2 per cent monocytes. A blood culture taken on 2-29-40 was reported 3-4-40 as positive for paratyphoid B. Because of this and because it was felt that if he had true leukemia relapse would recur, treatment with myelokentric acid was discontinued. Blood transfusions were not necessary for a period of two months. The spleen and liver had reduced in size clinically as well as radiographically. Furuncles and abscesses were almost entirely healed and he was up and about the ward. A bone marrow aspiration 3-28-40 revealed partial regeneration of the myeloid elements (fig. 2). He remained in good condition, but on 4-1-40, 4 per cent lymphoblasts were found in his peripheral blood. On this date an injection of inactive neutral fraction of urine extract was given and similar injections were given daily for three and one-half weeks. This material was given in 1½ cc. alkaline water emulsion. (One cc. was equal to 1,000 cc. urine.) During this injection period his relapse increased. There was an increased size of lymph nodes and spleen, and a shift to 50 per cent lymphoblasts in a



FIG. 1. MATERIAL FROM BONE MARROW ASPIRATION PRIOR TO TREATMENT, CASE 2.  
WRIGHT'S STAIN  $\times 900$

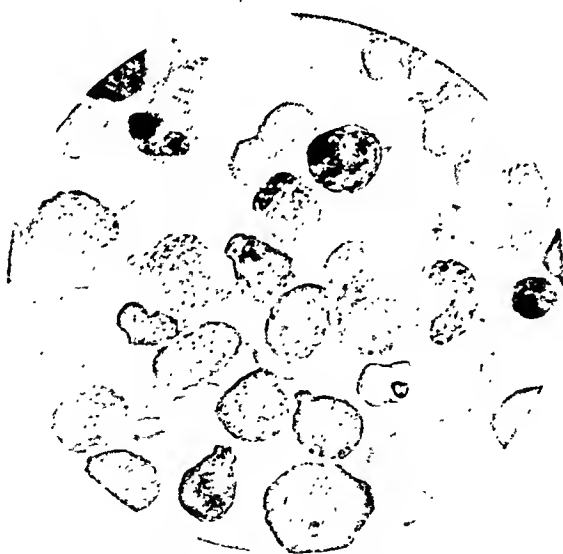


FIG. 2. MATERIAL FROM BONE MARROW ASPIRATION FOLLOWING TREATMENT, CASE 2.  
WRIGHT'S STAIN  $\times 900$

leukocyte count of 8,000 on 4-27-40. He again had fever, and blood transfusions were again necessary because of the recurrence of anemia. Injections of crude active myelokentric acid were again started on 4-24-40. These were continued daily for four weeks and a second remission began. There was again a marked drop in the leukocyte count to 1,100 cells and diminution in the percentage and number of lym-

phoblasts. There was also a reduction in spleen size and improvement in his clinical condition. From the end of May 1940 until the first of July he was given the inactive neutral fraction of urine extract and a third relapse period developed. On 7-1-40 he was given active crude material and this was continued until he died 8-19-40. Throughout the last month of his illness evidences of his leukemia were present at all times, as was evidence of his infection with paratyphoid B organisms. At this time sulfadiazine was given in an attempt to combat the infection. In the last two weeks of his life he developed a mediastinitis from which was cultured the paratyphoid organism. The leukocyte count the day before death was 12,000 with 45 per cent lymphoblasts. Unfortunately, permission for a necropsy was not obtained.

*Case 3.* E. S., a 9 year old white boy, entered the Jefferson Hospital 8-28-40 because of a "lump" on the side of his neck. This had been present for one month. He had lost 3 pounds in weight and had had a low grade fever for two weeks. All peripheral lymph nodes, the liver, and spleen were enlarged. The leukocyte count was 11,000, differential count was 6 per cent polymorphonuclear neutrophils, 13 per cent lymphocytes, 7 per cent metamyelocytes, 74 per cent lymphoblasts. The erythrocyte count was 3,400,000, hemoglobin 61 per cent, and the platelets were not reduced. A sternal marrow aspiration revealed a very hyperplastic marrow with 90 per cent lymphoid cells, and of these 60 per cent were lymphoblasts. The leukocyte count on 9-3-40 was 23,000 with the same differential count and on this day an initial dose of 2 cc. of crude myelokentric acid was given to him by intramuscular injection. One cc. of this material was equal to 500 cc. of urine. This was the chloroform extract of hydrolyzed urine and it was made up in alkaline water solution at a pH of 7.5. This amount was given daily for eleven days. At this time the dose was increased so that 1 cc. of extract was equal to 750 cc. of urine. Two cc. were given daily until 10-20-40, when a further increase in the dose was made so that he received 3 to 3½ cc., each cc. of which was equal to 1,000 cc. of urine. This latter dose was continued until 12-12-40 and after this date the extract was made up into capsules. These were given orally, fifteen each day, and this dose was equal to 2,800 cc. of urine. This was continued until 1-5-41.

On 9-13-40 the leukocyte count had risen to 44,000 but the percentage of lymphoblasts was reduced to 52. Slowly the leukocyte count fell and on 10-7-40 it was 9,000. The lymphoblasts had decreased to 24 per cent. At this time there was little decrease in the size of the lymph nodes, spleen, or liver, and clinically, improvement was slight. He was discharged from the hospital on 9-30-40 but injections were continued while he was at home. He was readmitted to the hospital 11-12-40 and was given three blood transfusions. On 11-26-40 the lowest leukocyte count was reached; it was 4,500. The differential count at this time was, polymorphonuclear neutrophils 6 per cent, lymphocytes 55 per cent, metamyelocytes 13 per cent, myelocytes 4 per cent, lymphoblasts 22 per cent. He was discharged from the hospital again on 11-26-40 and was cared for at home. In January, when treatment with the extract was discontinued, the leukocyte count was 17,000, erythrocyte count was 2,500,000, and the hemoglobin was 49 per cent. The differential count was polymorphonuclear neutrophils 5 per cent, eosinophiles 1 per cent, lymphocytes 49 per cent, metamyelocytes 8 per cent, lymphoblasts 37 per cent. No further therapy was given, including transfusions, but he was observed until death on 3-10-41. Permission for a necropsy was not obtained.

*Case 4.* R. W., a 2½ year old white girl, entered Jefferson Hospital 6-8-42. She had been ill for two weeks during which time her mother had noticed pallor, black and blue spots on her skin, and a loss of appetite. The spleen was enlarged 2 centimeters below the costal margin, the liver was slightly enlarged, and there was generalized lymphadenopathy. On 6-10-42 the erythrocyte count was 3,300,000, hemoglobin 50 per cent, the leukocyte count was 75,000, and the differential count was polymorphonuclear neutrophils 2 per cent, lymphocytes 53 per cent, monocytes 5 per cent, lymphoblasts 40 per cent. With the exception of one period, i.e., from August 6 to August 21, this child was given various extracts and fractions of extracts of feces from patients with chronic myeloid leukemia. She was given ½ cc. to 1 cc. injection daily, usually in oil of which the fecal equivalent was about 75 to 100 grams. During the period of August 6 to August 21 she was given ½ cc. to 1 cc. of urinary extract daily, 1 cc. equaling 1,000 cc. of urine. The fecal extracts were made up every two weeks; their potency was not known. The marrow from a bone marrow aspiration prior to treatment consisted almost entirely of lymphoblasts. The first twelve days that she was in the hospital the leukocyte count fell to 1,800 and the lymphoblasts completely disappeared from the blood stream. A second bone marrow aspiration at this time revealed an increase in myeloid elements in the marrow. The platelets increased in the blood stream from 56,000 at entry to 146,000 on 6-23-42.

The lymph nodes, spleen, and liver reduced in size and she was clinically improved. On 6-27-42 her peripheral blood showed 30 per cent myeloid white cells, 68 per cent lymphocytes, and 2 per cent monocytes and no lymphoblasts. On July 6, however, her leukocyte count had increased to 40,000 with 16 per cent polymorphonuclear neutrophils, 82 per cent lymphocytes and 2 per cent lymphoblasts. Through July and into August her leukocyte count varied from 22,000 to 71,000 with large numbers of lymphocytes and only a few lymphoblasts. During the period in which she received urinary extract the leukocyte count remained high. The week after this was discontinued, however, and fecal extract was again used the leukocyte count decreased to 800 cells with 92 per cent lymphocytes. The leukocyte count then rose to 4,300, and on 9-16-42 the reticulocyte count was 6.5 per cent and the platelet count was 100,000. At this time there were 49 per cent polymorphonuclear neutrophils in the peripheral blood.

She again relapsed completely and the leukocyte count rose to 156,000 but contained only a small number of lymphoblasts—2 per cent. The lymph nodes and spleen remained reduced in size throughout the entire course of her illness after treatment was started. She died 10-10-42. The leukocyte count the day of death was 300. At this time she had a papular eruption over her entire body which might have been due to septicemia, but no blood culture was taken. A necropsy was performed.

*Case 5.* L. R., a 2½ year old white girl, entered the hospital 7-12-44. She had lost 2 to 3 pounds in weight, was pale, and had had anorexia for six weeks before coming to Jefferson Hospital. She had been in another hospital the two weeks before entry at Jefferson and had received two blood transfusions. The spleen and all peripheral lymph nodes were moderately enlarged and the leukocyte count was 15,000, of which 59 per cent were lymphocytes, 40 per cent were lymphoblasts, and 1 per cent were polymorphonuclear neutrophils. The erythrocyte count was 2,600,000, hemoglobin 48 per cent, and the platelet count was 22,000. A bone marrow aspiration revealed marrow made up almost entirely of lymphoblasts. For eleven days she was given 10 cc. or 100 units daily of adrenal cortical extract. This was done in order that we might have a period of control before the urinary extracts were administered. Throughout this period there was no improvement clinically nor was there a change in her blood cell elements. The period between 7-25-44 and 8-22-44, or for twenty-four days, she received ½ cc. to 1 cc. of urinary extract daily (1 cc. of which was equal to 4,000 cc. of urine). This material was the chloroform extract of the hydrolyzed alcoholic eluate from kaolin adsorbate of urine. The larger dose was used because this was not as potent as the chloroform extracts of hydrolyzed urine. Because of a lack of urine from August 22 to 28 she received the extract from only 2,500 cc. of urine daily, but after that the dose was again increased to 6,000 or even 12,000 cc. daily. On 7-24-44 the leukocyte count was 21,000 with 87 per cent of the cells lymphoblasts, after which it gradually fell so that on August 5 it was 3,300 and the lymphoblasts were only 24 per cent. On August 17 the leukocyte count was 5,000; the differential count was 9 per cent polymorphonuclear neutrophils, 1 per cent eosinophiles, 2 per cent myelocytes, 88 per cent lymphocytes, and 0 lymphoblasts. Throughout this period her erythrocyte count was maintained at or above 3,000,000 without transfusions of blood. However, she gradually slipped into relapse again and on September 4 had 78 per cent lymphoblasts in a leukocyte count of 60,000. On September 17, and 18, and 20 she was given a total of 300 r of x-ray over neck, thymus, and spleen with the hope that this plus the urinary extracts would control the leukemic process. The leukocyte count then fell to, and remained under, 1,000 from September 25 to October 2, the day of her death. A middle ear infection was controlled during her hospitalization by penicillin.

In the last week of her illness she developed an abscess of her right buttock and penicillin was given to control this. She died 10-2-44 and a necropsy was performed.

*Case 6.* D. H., a 4 year old white boy, had been ill for five months when he entered Jefferson Hospital 9-26-45. He tired easily and had had anorexia throughout this time. He was irritable, pale, and had evidently lost a little weight. The axillary, cervical, and inguinal lymph nodes were enlarged. The abdomen was protuberant because of enlargement of the spleen and liver. The erythrocyte count was 810,000 and the leukocyte count was 9,500. The platelet count was 30,000, and the differential count revealed 41 per cent lymphocytes and 59 per cent lymphoblasts. He was given a blood transfusion and injections of urinary extract were begun. This extract was the hydrolyzed eluate from kaolin adsorbed urine. The first twenty-five days he received material daily from the same lot of extract. Each injection was equal to from 5,000 cc. to 20,000 cc. of urine. From September 26 to October 17 he received four blood transfusions. The

leukocyte count slowly decreased until on October 13 it had reached 1,500. Lymphoblasts at this time were only 12 per cent of the total count. The lymph nodes and spleen became smaller and he began to have a better appetite. On October 12 he had a sore throat, for which penicillin was given. After his throat became well the leukocyte count gradually increased to between 3,000 and 5,000 but the lymphoblasts remained low in number. The platelet count increased to 54,000 and the erythrocyte count was maintained at 3,000,000 without a blood transfusion for five weeks.

He was given injections of the same type of extract from a different lot beginning 10-17-45, but he received only an amount equal to 2,500 cc. of urine daily. This was continued for sixteen days and was then doubled in amount for six days, but on 11-15-45 chloroform extract similar to that given to cases 1, 2, and 3 was administered in doses of 1 cc. daily (each cc. was equal to 1,000 cc. of urine). This was continued until seven days before his death. During the last three weeks of his life he was obviously in relapse, with a leukocyte count as high as 35,000 cells, of which 81 per cent were lymphoblasts. The leukocyte count, however, fell during the last week of his life and was 2,700 the day of his death. He had developed an abscess of the left buttock which was incised and following this he bled from the wound and from the nose and throat. *Staphylococcus aureus* was cultured from the abscess. Death occurred on 12-2-45 and a necropsy was performed.

*Case 7.* A. B., an 11 year old white boy, entered Jefferson Hospital 12-14-45. He had been ill for one year. His illness started with joint pains and fever. In March 1945 a diagnosis of leukemia was made because of a leukocyte count of 20,000 which contained large numbers of lymphocytes and lymphoblasts. At that time his erythrocyte and platelet counts were low. Throughout the year he had received at least one transfusion of blood a week. The leukocyte count at one time was nearly 100,000. He had several attacks of middle ear infection and many attacks of joint pains simulating acute rheumatic fever. He had lost a great deal of weight and weighed only 40 pounds when admitted to the hospital. On 12-14-45 the leukocyte count was 59,000, and the erythrocyte count was 1,800,000, with hemoglobin of 35 per cent and the platelet count was 10,000. The differential count was polymorphonuclear neutrophils 1 per cent, myelocytes 1 per cent, small lymphocytes 6 per cent and lymphoblasts 92 per cent. A bone marrow aspiration had been done elsewhere and the marrow was made up largely of lymphoblasts although it was not very cellular. He was treated with chloroform extract of urine (1 cc. of which equaled 1,200 cc. of urine) administered by intramuscular injection in doses of  $\frac{1}{2}$  cc. daily for nineteen consecutive days. The last two days of his life this was not given. Along with this he received 3 units of liver extract daily and a total of five transfusions of blood. He had a middle ear infection which yielded to treatment with penicillin. The leukocyte count decreased to 2,600 the day of death but the percentage of lymphoblasts remained at about 90. Six days before death he passed bloody urine and from then on he bled from the urinary tract, from the nose, and from the bowel. He died 1-7-46 and a necropsy was performed.

*Case 8.* R. H., a 14 year old white boy, entered Jefferson Hospital 5-2-46. For four months he had complained of easy fatigue and a loss of weight. For about four years he had been using lacquer and quick-drying airplane glue in building model airplanes. These probably contained benzol. In December 1945 he had had a severe "cold" and since that time had had weakness, joint pains, nosebleeds, and anorexia. At another hospital during a six week period he received twelve blood transfusions. At that time a bone marrow aspiration had shown a marrow that was very cellular and contained large numbers of lymphoblasts.

On entry to Jefferson Hospital examination disclosed the spleen and liver to be palpable at the rib margins, and there was generalized slight lymphadenopathy and pallor. The erythrocyte count was 2,600,000, hemoglobin 59 per cent, and the leukocyte count was 6,300, and the platelet count was 10,000. The differential count was polymorphonuclear neutrophils 10 per cent, lymphocytes 55 per cent, lymphoblasts 25 per cent, monocytes 10 per cent. The sternal marrow aspiration was repeated and it revealed over 90 per cent lymphocytes and lymphoblasts in a very cellular marrow (fig. 3). On 5-2-46 he was given his first injection of crude myelokentric acid. This was a chloroform extract of hydrolyzed urine made up in alkaline water solution at a pH of 7.5 (1 cc. was equal to 1,000 cc. of urine). At first he was given 1 cc. daily. He had averaged 1 cc. daily throughout the entire treatment period up to August 3 although in the months of June and July the material was given two to four days apart in 2 to 4 cc. injection doses. At first he had a fever of 101° to 104° F. daily and because of a cough and rhinitis he was given penicillin by

injection for two weeks. The erythrocyte count was maintained by blood transfusion. The first month it was necessary to give a blood transfusion every four or five days, but after June 1 the intervals between transfusions were ten days to two weeks. The leukocyte count dropped slowly until it reached a low point of 1,400. As the total count fell the lymphoblasts dropped steadily in percentage and actual numbers. Gradually the leukocyte count began to return to normal levels, and as it did normal lymphocytes and polymorphonuclear leukocytes made up the greater part of the differential count. On June 22 the erythrocyte count was 2,600,000, hemoglobin 54 per cent, the leukocyte count was 3,500, and the platelet count was 138,000. The differential count was polymorphonuclear neutrophils 48 per cent, lymphocytes 50 per cent, lymphoblasts 2 per cent. Another bone marrow aspiration was done on June 18, and it revealed a very hyperactive marrow with about one-third of the cells myeloid in character including red cell and platelet formation (fig. 4). The liver, spleen, and lymph nodes all were reduced somewhat in size. He became more active and his appetite was very good. June 28 he was discharged to be observed outside of the hospital. From then until this writing, 8-3-46, he has remained in partial remission.

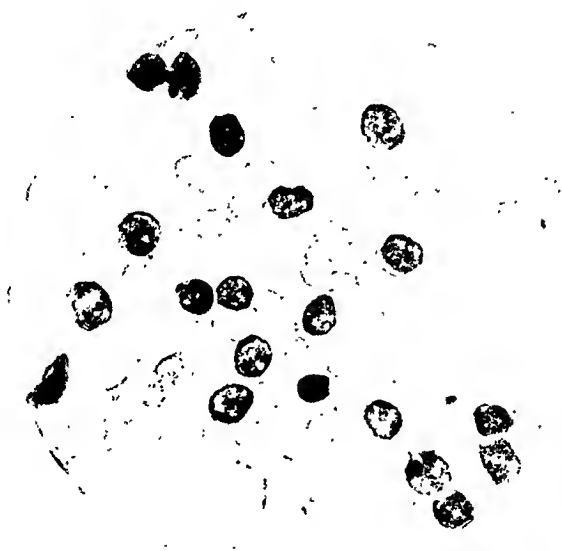


FIG. 3. MATERIAL FROM BONE MARROW ASPIRATION PRIOR TO TREATMENT, CASE 8.  
WRIGHT'S STAIN  $\times 900$

Case 9. A. C., a 30 year old white man, had been ill for about ten weeks when he entered Jefferson Hospital 1-16-40. He had had night sweats, some fever, had lost 17 or 18 pounds in weight, and had noted four weeks before entry that the lymph nodes in his neck were swollen. Ten days before coming to the hospital his vision was blurred in the right eye. On examination there was a hemorrhage in the retina and evidence of recent bleeding from both nostrils; there was generalized enlargement of the peripheral lymph nodes, and the spleen was enlarged about 2 centimeters below the costal margin. The hemoglobin was 69 per cent, erythrocyte count was 3,000,000, and the leukocyte count was 26,000. The platelet count was 70,000 and the differential count was polymorphonuclear neutrophils 12 per cent, lymphocytes 74 per cent, myelocytes 10 per cent, lymphoblasts 4 per cent. A bone marrow aspiration revealed a cellular marrow made up largely of lymphoblasts. He was given a total of 1,000 r of x-ray over neck, chest, and groins. His temperature was 102° F. to 104° F. daily. He developed oozing of blood from nose and gums and many petechiae over the entire body. The leukocyte count fell to 5,000 1-28-40 but rapidly rose to 110,000 the day before he died. He died 2-16-40 and a necropsy was performed.

*Case 10.* R. S., a 3½ year old white boy, entered Jefferson Hospital 6-17-42 because of pallor and a distended abdomen. He was well until 3-1-40, at which time he had had a "cold." Shortly after that, because of pallor and distention of the abdomen, he was taken to a hospital where the diagnosis of leukemia was made. Prior to admission to Jefferson Hospital he had had several transfusions. His erythrocyte count was maintained, with difficulty, at around 3,000,000. His leukocyte count varied from 15,000 to 35,000 and the cells in the peripheral blood were all termed lymphocytes.

In Jefferson Hospital it was found that all of the peripheral lymph nodes were moderately enlarged. The abdomen was found to be large because of the size of the spleen and liver. The erythrocyte count was 1,200,000, hemoglobin 34 per cent, leukocyte count was 107,000 and the platelet count was 44,000. The differential count was lymphocytes 65 per cent, lymphoblasts 35 per cent. He was given five transfusions, and the last two days of his life he was given two injections of the inactive neutral fraction of extract from feces. The erythrocyte count was 1,400,000, hemoglobin 29 per cent, and the leukocyte count was

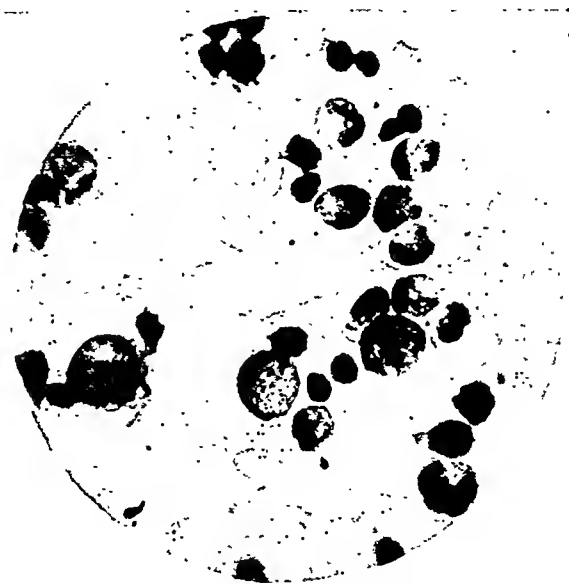


FIG. 4. MATERIAL FROM BONE MARROW ASPIRATION AFTER 6 WEEKS OF TREATMENT, CASE 8.  
WRIGHT'S STAIN  $\times 900$

5,000 the day of death with no change in the differential count. The day of death it was found that he had an abscess of his right buttock. He died 6-29-42; a necropsy was performed.

*Case 11.* H. S., a 16 year old boy, entered Jefferson Hospital 4-14-45 because of pains in the muscles of arms and legs, loss of weight, and fever for a period of four months. He had been in another hospital in January 1945 and was thought to have rheumatic fever. At that time it was found that he had anemia and two blood transfusions were given. He was also given penicillin because the spleen was enlarged and he had a fever of 102° to 103° F. daily. When he entered Jefferson Hospital it was obvious that he had lost weight. At this time the spleen and liver were palpable, there was slight enlargement of the inguinal lymph nodes, but other lymph nodes were not enlarged. The sternum and the bones of the legs were tender to pressure.

X-ray examination of the chest showed nothing abnormal, but examination of the legs showed an elevation of the periosteum of each fibula, more on the left than on the right. A sternal marrow aspiration revealed a very hyperplastic marrow, the cells of which were preponderantly lymphoblasts. At this time his peripheral blood was not particularly abnormal. On 4-19-45 the erythrocyte count was 3,700,000, with

a hemoglobin of 69 per cent. The leukocyte count was 7,900 with a differential count of polymorphonuclear neutrophils 64 per cent, eosinophiles 1 per cent, basophiles 1 per cent, lymphocytes 27 per cent, monocytes 6 per cent, lymphoblasts 1 per cent. A biopsy of the fibular marrow revealed a lymphoid reaction with infiltration of lymphoid cells into the cortical bone.

The peripheral blood gradually changed so that on 5-18-45 in 15,000 leukocytes 42 per cent lymphoblasts were present. He became more anemic and it was necessary to give him blood transfusions. He also had an infection of his right eye which was controlled with penicillin. On 6-5-45 his leukocyte count was 24,000 and of these 77 per cent were lymphoblasts. Late in the disease he bled from the urinary tract, bowels, and nose. The day before death the leukocyte count was 108,000 with 82 per cent lymphoblasts. He died 6-9-45 and a necropsy was performed.

*Case 12.* S. W., an 8 month old colored girl, one of twins, entered Jefferson Hospital 4-17-45 because of swelling of the eyelids, irritability, and anorexia. There was a generalized lymphadenopathy, the eyelids were swollen, the mucous membranes were pale, and the liver enlarged. She had been ill for about two weeks. The erythrocyte count was 1,700,000 with a hemoglobin of 21 per cent, the leukocyte count was 38,000, and the differential count was polymorphonuclear neutrophils 4 per cent, lymphocytes 4 per cent, and lymphoblasts 92 per cent. Her platelet count was 4,000. The leukocyte count fell in two days to 5,000. Transfusion of blood was given but she died 4-19-45 and a necropsy was performed.

#### PATHOLOGY

Grossly there were no clear-cut variations between the treated and untreated cases. Petechiae and hemorrhages were present in the skin of 2 of the controls and in 3 of the treated, and in the pericardium of 1 of the former and of 3 of the latter. In both groups there was generalized enlargement of the lymph nodes to a maximum of 2.5 cm. in diameter. Making an allowance for the difference in ages, the spleens of the untreated children (weighing 170 Gm. in case 9 and 90 Gm. in case 12) were definitely larger than were those of the treated ones (weighing 120 Gm. in case 5, 50 Gm. in case 4, 120 Gm. in case 1, and 90 Gm. in case 6, and 150 Gm. in case 7). All livers were enlarged but they showed no appreciable change in size and appearance in the two groups. The involvement of the kidneys was more extensive in control cases 9, 11, and 12 (one kidney in each weighing 320 Gm., 670 Gm., and 90 Gm. respectively) than it was in the treated cases 1, 4, 5, 6, and 7 (whose corresponding weights were 130 Gm., 150 Gm., 110 Gm., 55 Gm., and 90 Gm. respectively). The cortices in each were swollen and pale brown and exhibited irregular petechiae and larger blotchy areas of erythrocytic extravasation. Hemorrhages in the lungs, prominence of the lymphoid patches in the intestines, and the appearance of the bone marrow were the same in both groups.

*Microscopic:* Histologic sections of all organs were stained with hematoxylin and eosin and in addition sections of the lymph nodes, liver, spleen, and bone marrow were stained with Foote's reticulum stain, Giemsa's stain, and Masson's trichrome stain.

*Lymph Nodes:* In both the control and treated cases the normal architecture was almost completely effaced and the capsules were infiltrated with leukemic cells. In the controls there was a diffuse and dense or moderate infiltration with lymphoblasts and lymphocytes and almost no pleomorphism of cells (fig. 5). In the background there were sparse reticulum cells, occasional polymorphonuclear leukocytes, and no phagocytes. In case 12 the vessels were quite prominent and there was a slight increase of perivascular connective tissue but in none of the cases was there reticulum hyperplasia.

In contrast, the lymph nodes of the treated cases disclosed (1) an increase in vascularity with sometimes a surrounding zone of necrosis, (2) reticulum hyperplasia, and (3) an unmistakable pleomorphism of cells. The vessels were thick-

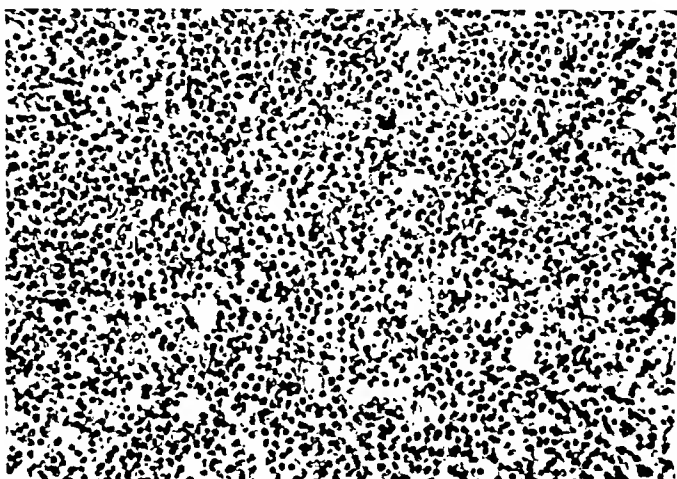


FIG. 5. SECTION OF A LYMPH NODE FROM AN UNTREATED CASE SHOWING A UNIFORM INFILTRATION WITH LYMPHOID CELLS. HEMATOXYLIN AND EOSIN  $\times 200$

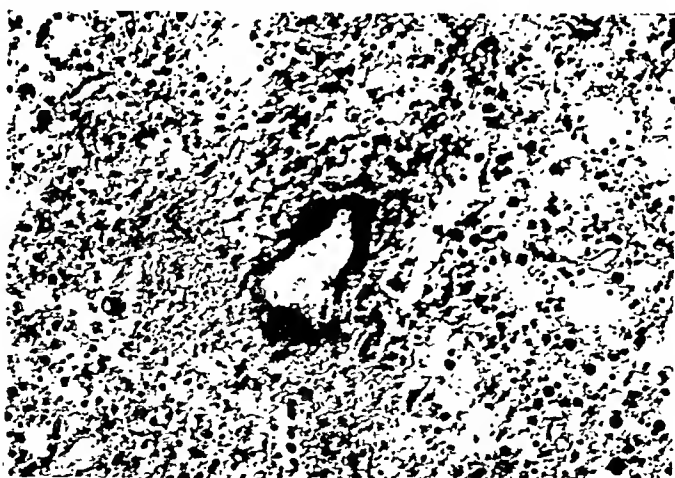


FIG. 6. SECTION OF A LYMPH NODE FROM A TREATED CASE SHOWING NECROSIS OF A VESSEL AND ITS IMMEDIATELY SURROUNDING TISSUE. NOTE THE PLEOMORPHISM OF THE MORE PERIPHERAL CELLS. HEMATOXYLIN AND EOSIN  $\times 200$

walled capillaries often distended with erythrocytes. Their outlines were usually distinct but sometimes they merged directly with a surrounding zone of necrosis (fig. 6). The reticulum cells were either diffusely increased or formed conspicuous

bands of cells radiating peripherally from the medulla (figs. 7, 8, and 9). The cells were large, polygonal or irregular in shape, and contained round evenly stained nuclei and an abundant amount of pink cytoplasm with processes often attached to the underlying reticulum. While the infiltrating cells were mostly lymphocytes

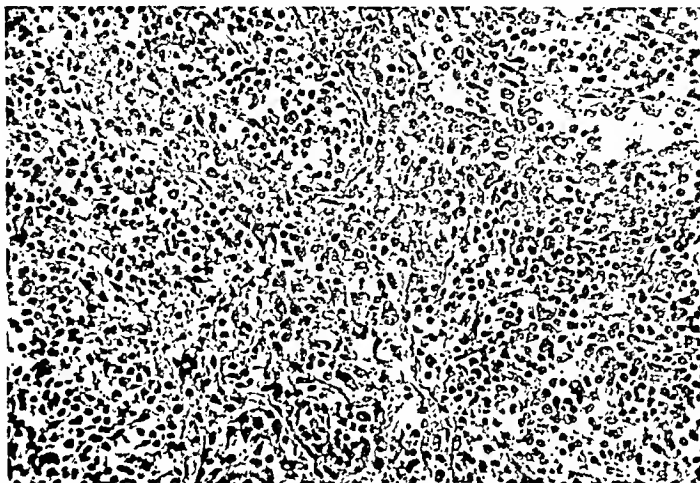


FIG. 7. SECTION OF A LYMPH NODE FROM A TREATED CASE SHOWING A MARKED INCREASE IN RETICULUM CELLS. HEMATOXYLIN AND EOSIN  $\times 200$



FIG. 8. SECTION OF A LYMPH NODE FROM AN UNTREATED CASE SHOWING A PAUCITY OF RETICULUM. FOOTE'S RETICULUM STAIN  $\times 200$

and lymphoblasts, the pleomorphism was sometimes so marked and of such a nature as to suggest a more than striking resemblance to Hodgkin's disease (fig. 10). The most conspicuous elements perhaps were very irregularly shaped cells of an over-cell size equal to that of lymphocytes or lymphoblasts. They contained almost

imperceptible cytoplasm and darkly stained nuclei, and were undoubtedly distorted lymphocytoid cells. In addition to these cells there were scattered phagocytes, polymorphonuclear leukocytes, eosinophiles, plasma cells, and large mononuclear or multinuclear cells resembling Sternberg-Reed cells. Some of the



FIG. 9. SECTION OF A LYMPH NODE FROM A TREATED CASE SHOWING A MARKED INCREASE IN RETICULUM. FOOTE'S RETICULUM STAIN  $\times 200$

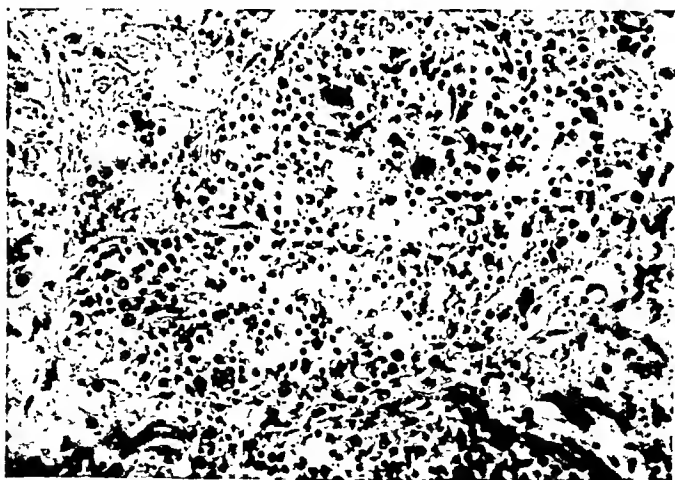


FIG. 10. SECTION OF A LYMPH NODE FROM A TREATED CASE SHOWING A MARKED PLEOMORPHISM OF CELLS RESEMBLING HODGKIN'S DISEASE. GIEMSA STAIN  $\times 200$

nodes also disclosed a diffuse, early fibrosis. Sections of the thymus from case 6 showed a reticulum cell hyperplasia and a pleomorphism of cells similar to that seen in the nodes of the same case.

*Liver:* The leukemic infiltrates in the livers of the control cases were prominent

throughout the portal radicles and very sparse between the liver cords (fig. 11). In cases 9, 10, and 11 all the extraneous cells were lymphoblasts or lymphocytes, whereas in case 12 there were also a few distorted cells in the portal radicles. Con-

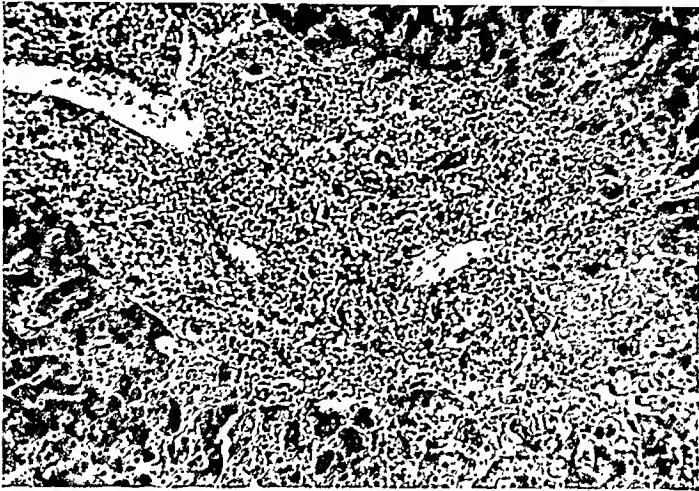


FIG. 11. SECTION OF A LIVER FROM AN UNTREATED CASE SHOWING MASSIVE PORTAL INFILTRATION WITH LYMPHOID CELLS. HEMATOXYLIN AND EOSIN  $\times 100$

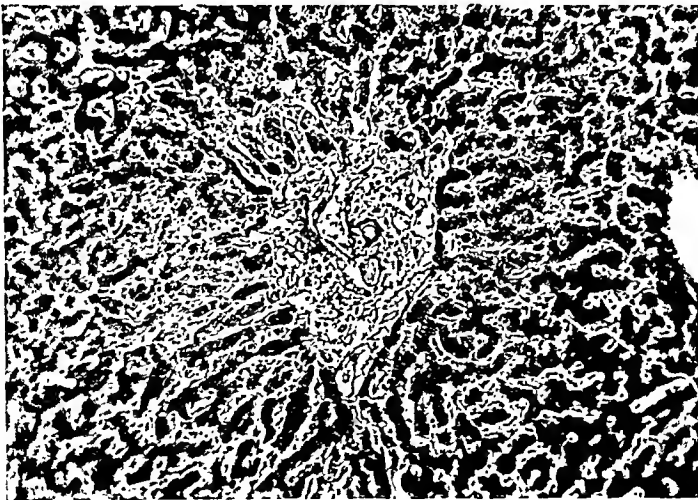


FIG. 12. SECTION OF A LIVER FROM A TREATED CASE SHOWING A PORTAL RADICLE FROM WHICH THE LEUKEMIC CELLS HAVE BEEN ALMOST COMPLETELY WASHED AWAY LEAVING A NAKED SCANTY UNDERLYING RETICULUM. HEMATOXYLIN AND EOSIN  $\times 100$

nective tissue cells were slightly increased in the latter location in case 12 but were not evident in cases 9, 10, and 11, and Kupffer's cells were difficult to demonstrate in any of the controls. The distribution of the leukemic cells in the livers from the

treated cases was essentially the same as that in those from the controls. The former, however, differed from the latter in that there was (1) a definite pleomorphism of cells similar to that already described in the lymph nodes, (2) a hyperplasia of reticulum cells, (3) an increase in reticulum and a corresponding depletion



FIG. 13. SECTION OF A LIVER FROM AN UNTREATED CASE SHOWING A RELATIVELY SCANTY RETICULUM IN A PORTAL RADICLE. FOOTE'S RETICULUM STAIN  $\times 200$

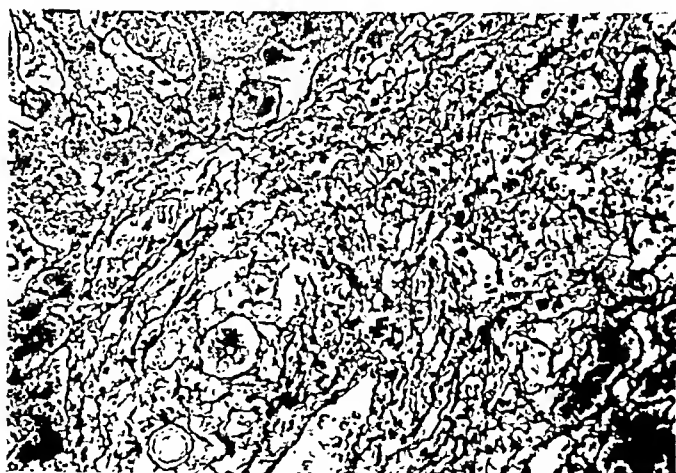


FIG. 14. SECTION OF A LIVER FROM A TREATED CASE SHOWING AN INCREASE IN RETICULUM IN A PORTAL RADICLE. FOOTE'S RETICULUM STAIN  $\times 200$

of lymphoid cells (figs. 12, 13, and 14), and (4) a swelling, bulging, and what even appeared to be a detachment of some of the Kupffer's cells into the sinusoidal lumens (fig. 15). Except for some increase of fat droplets in the liver cells, the hepatic structure was otherwise unchanged.

*Spleen:* Sections of the spleens from the controls showed a few distorted follicles remaining in case 12 and a complete effacement of the normal architecture in cases 9, 10, and 11 (fig. 16). In the latter the cells were almost all of the lymphocytic type, but in case 12 there were also a few phagocytes and occasional polymorpho-



FIG. 15. SECTION OF A LIVER FROM A TREATED CASE SHOWING VERY PROMINENT KUPFFER'S CELLS, SOME OF WHICH APPEAR TO BE EXTRUDING INTO THE LUMEN. HEMATOXYLIN AND EOSIN  $\times 400$

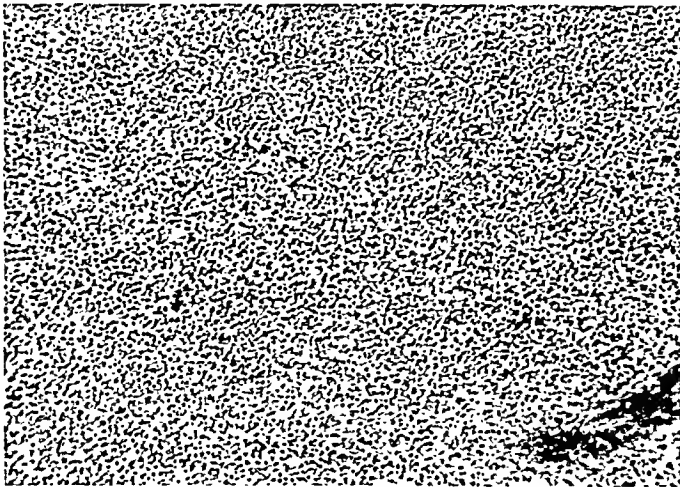


FIG. 16. SECTION OF A SPLEEN FROM AN UNTREATED CASE SHOWING A COMPLETE EFFACEMENT OF THE NORMAL ARCHITECTURE AND A DIFFUSE INFILTRATION WITH LYMPHOID CELLS. HEMATOXYLIN AND EOSIN  $\times 100$

nuclear leukocytes and eosinophiles. In this case the sinuses and reticulum were discernible, although not hyperplastic, while in cases 9 and 10 they were inconspicuous. Sections of the spleens from the treated cases showed the same topographical changes as did those from the untreated ones. As in the other organs they

differed from the latter in that there was (1) a focal or diffuse increase in reticulum (figs. 17 and 18), (2) a prominence of the sinusoids, and (3) a marked pleomorphism of cells. In case 6 some of the foci of reticulum hyperplasia disclosed early necrosis but in the remaining cases such degenerative changes were not encountered. In case

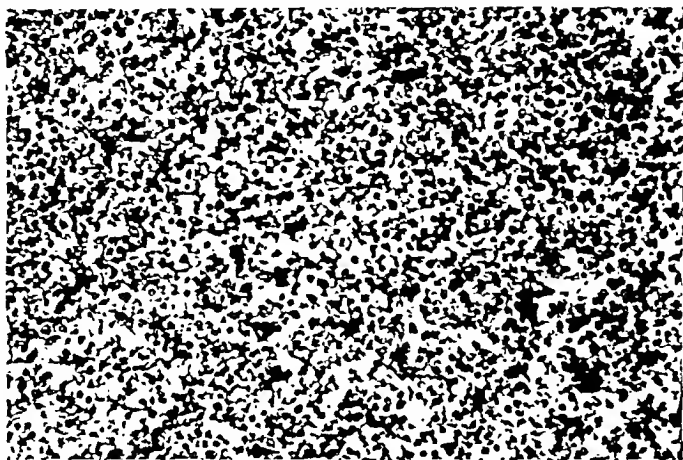


FIG. 17. SECTION OF A SPLEEN FROM AN UNTREATED CASE SHOWING A PAUCITY OF RETICULUM.  
FOOTE'S RETICULUM STAIN  $\times 200$

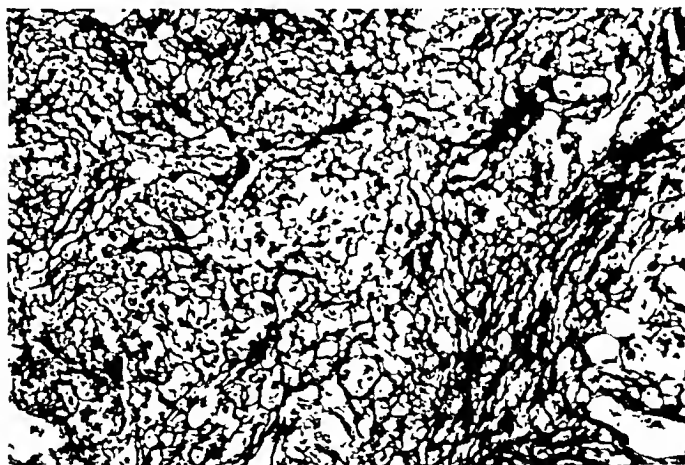


FIG. 18. SECTION OF A SPLEEN FROM A TREATED CASE SHOWING A MARKED INCREASE OF RETICULUM.  
FOOTE'S RETICULUM STAIN  $\times 200$

1, whose spleen was removed before treatment, there was no increase in reticulum and no such pleomorphism of cells as was seen in the spleens of treated cases. In all treated cases although most of the infiltrating cells were lymphoblasts and lymphocytes there were also varying numbers of phagocytes, reticulum cells, monocytes,

plasma cells, polymorphonuclear leukocytes, eosinophiles, and large mononuclear or binuclear cells resembling Sternberg-Reed cells (fig. 19).

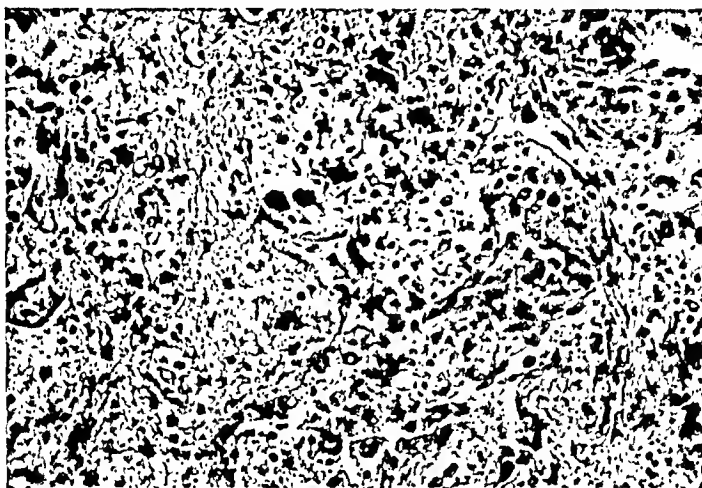


FIG. 19. SECTION OF A SPLEEN FROM A TREATED CASE SHOWING A PLEOMORPHISM OF CELLS THAT RESEMBLES HODGKIN'S DISEASE. HEMATOXYLIN AND EOSIN  $\times 200$

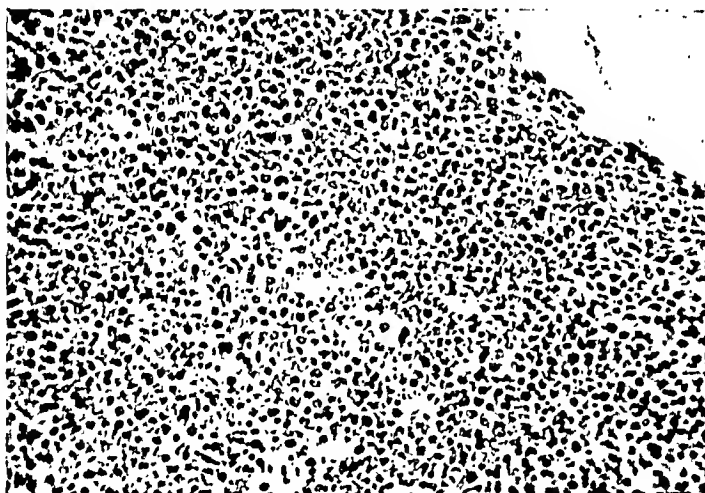


FIG. 20. SECTION OF BONE MARROW FROM AN UNTREATED CASE SHOWING A DIFFUSE AND DENSE INFILTRATION WITH LYMPHOID CELLS. HEMATOXYLIN AND EOSIN  $\times 200$

*Bone Marrow:* Sections of sternal and vertebral bone marrow were studied histologically. In the control cases it was diffusely involved, showing a moderate or dense infiltration with leukemic cells (fig. 20). These composed from 75 per cent to 90 per cent of all the marrow elements with the remaining constituents consisting of megakaryocytes and cells of the erythrocytic and myelocytic series. There was

osteosclerosis in case 10 but in none of the cases was there reticulum cell hyperplasia or fibrosis. Sections of the marrow from the treated cases also showed a diffuse involvement but, in contrast to those of the control cases, it was less cellular and distinctly more "washed out" (fig. 21). About one-half of the infiltrating cells



FIG. 21. SECTION OF BONE MARROW FROM A TREATED CASE SHOWING A SEVERE DEPLETION OF ALL CELLS  
HEMATOXYLIN AND EOSIN  $\times 100$

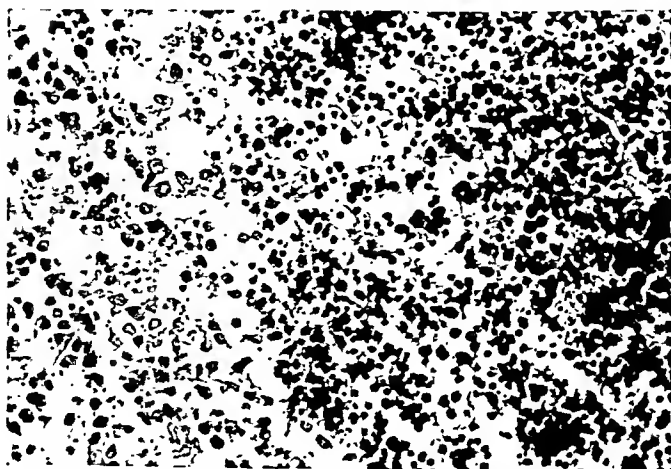


FIG. 22. SECTION OF BONE MARROW FROM A TREATED CASE SHOWING A DECREASE IN CELLULARITY AND A  
MARKER PLEOMORPHISM OF CELLS. HEMATOXYLIN AND EOSIN  $\times 200$

were lymphoblasts and lymphocytes whereas the remainder was composed of reticulum cells, large primitive cells resembling Sternberg-Reed cells, plasma cells, few eosinophiles, polymorphonuclear leukocytes, and other unidentified cells (fig. 22). Throughout the sections there was a diffuse or focal increase of reticulum

to which processes of the reticulum cells were often attached (figs. 23 and 24). All sections showed almost a complete absence of megakaryocytes.

*Kidney:* The leukemic infiltrates in the kidneys were similar in distribution in both the control and treated cases. They were confined to the interstitial tissue of



FIG. 23. SECTION OF BONE MARROW FROM AN UNTREATED CASE SHOWING A PAUCITY OF RETICULUM.  
FOOTE'S RETICULUM STAIN  $\times 200$



FIG. 24. SECTION OF BONE MARROW FROM A TREATED CASE SHOWING A MARKED INCREASE IN RETICULUM.  
FOOTE'S RETICULUM STAIN  $\times 200$

the tubules and around the glomeruli, and varied from a few foci to severe infiltrations. In the untreated cases the cells were entirely of the lymphocytic type but in the treated ones there were also distorted cells, plasma cells, monocytes, occasional eosinophiles, and polymorphonuclear leukocytes. Erythrocytic extrav-

asation, although present in both groups, was much more severe in the treated cases.

*Other Organs:* In both the control and treated cases small foci of leukemic cells were found in the adrenals, lungs, heart, pancreas, and skin. Except for some of the pleomorphism already referred to, these foci were similar in both groups. There was a variety of cells in greatly hyperplastic lymph follicles of the intestine in both the treated and untreated cases and in each there was ulceration of the overlying epithelium. Congestion, edema, and hemorrhages were found with equal frequency and severity in the lungs of both groups of cases.

#### RECAPITULATION OF CLINICAL AND PATHOLOGICAL MATERIAL

The clinical part of this paper is presented in order that it may be seen that a trend toward normal blood levels was initiated in each of the treated cases. This was especially true in cases 1, 2, 4, and 8. To a lesser degree it was apparent in cases 3, 5, and 6, while in case 7 the trend was very slight.

In each case except case 1 there was a marked drop in total leukocyte count twelve days to six weeks following the first dose of crude myelokentric acid. The leukocyte counts of cases 2 and 4 reached levels under 1,000; those of cases 6 and 8 were reduced to under 2,000 cells. In case 1 the level of the leukocytes was under 2,000 when treatment was started. Case 5 reached a low count of 3,000 and case 3 a low count of 4,500. After the lowest count was reached in each case, with the exception of cases 3 and 7, the subsequent rise was accompanied by normal elements in the hemogram. As the leukocyte counts began to rise young erythrocytes and increased numbers of platelets were found in the peripheral blood. The bone marrow aspirations that were obtained after the leukocyte counts began to rise and normal elements had appeared in the peripheral blood of cases 1, 2, 4, and 8 revealed a regeneration of myeloid elements and a diminution of lymphoid cells as compared with the aspirations obtained prior to treatment. Five of the 8 cases had remissions so marked that transfusions were unnecessary for periods of from three weeks to two months. In cases 1, 2, 4, 5, and 6 during these periods the erythrocyte levels were maintained at or above 3,000,000 cells. Before treatment and throughout the first five weeks of treatment case 8 required transfusion not less than once a week and frequently every four to five days. As treatment progressed this case required transfusion only every ten days to two weeks. The size of lymph nodes, spleen, and liver was reduced in each case that received this treatment, although case 3 showed some fluctuation in the size of the lymph nodes irrespective of treatment.

The leukocyte count of case 9 fell from 26,000 to 5,000 in two weeks of observation, but again increased to 110,000 before death and there was no change in the organ pathology. The leukocyte count of case 10 fell from 107,000 to 5,000 in twelve days, but there was no change in the size of the spleen or liver or of the lymph nodes, and necropsy material showed no alteration from the usual leukemic process. A change from normal leukocyte levels with normal differential values to leukemic levels with large numbers of abnormal cells occurred in case 11 in a five week period, and of course the necropsy material was typical of lymphoid leukemia. The leukocyte level of case 12 dropped from 38,000 to 5,000 in two days, again without change in organ pathology.

The relationship of infection to the partial remissions reported here must be considered, because cases 2, 4, 5, and 6 each had severe infections. Cases 1, 3, 7, and 8, however, did not have severe infections and the remissions and necropsy material were much the same whether or not infection had been encountered. Aside from these facts 2 of the control cases had infection which did not alter the course or organ pathology in these cases. Others<sup>7</sup> also have remarked on the coincidence of infection in the leukemias of childhood, but in such reports it is rare to find a change in the pathology of the disease from the action of the infective agent. Case 5 (a treated case) and case 10 (a control) were each given small doses of x-ray. It seems unlikely that the alteration in organ pathology could be accounted for by the action of x-ray in case 5.

The length of life of all cases is shown in table 1. In the treated cases we have used the length of life from the start of treatment to death, and for the untreated cases from the start of our observations to death.

This is too small a series from which to draw any conclusions, but the table is given because of the striking difference between the two groups.

TABLE 1.—Length of Life in Treated and Control Cases

TREATED	CONTROL
Case 1—6½ months	Case 9—12 days
Case 2—8 months	Case 10—1 month
Case 3—6 months	Case 11—2 months
Case 4—4 months	Case 12—2 days
Case 5—1½ months	
Case 6—2 months	
Case 7—3 weeks	
Case 8—6½ months (still living)	

We believe that we have induced partial remissions in these 8 cases thirteen times. This number of remissions is obtained by adding the very definite changes that occurred in the blood picture and bone marrow and in the general condition of the patients to the changes that occurred toward the end of life in cases 1, 4, and 7. We believe that these latter were consistent with the necropsy findings.

The remissions were more complete in cases 1, 2, 4, and 8 than in the other cases. The necropsy material of case 7, however, represented a greater change from the usual leukemic process than did material from the other 4 in the treated necropsy group. It is interesting in this connection that to cases 1, 2, 7, and 8 liver extract (anti-anemia factor), 1 to 3 units, was given with each dose of crude myelokentric acid. A relationship between the action of the two substances might be suggested but further work is needed to be certain of this.

It must be remembered that all the material used was crude and because of the variations in its source great variations in potency occurred from one lot to another. Cases 5 and 6 might have gone further into remission except that at a crucial point in each case, because of lack of urine, it was necessary to reduce the dose of the extract. Then, too, patients may build up an increased tolerance to the material and need more of it the longer they are treated. It should be pointed out that 2 patients,

cases 1 and 2, were given inactive fractions of urine extracts for adequate periods of time with no effect on the blood picture or improvement in general condition.

The histopathologic changes in the various organs of the treated cases are in keeping with the clinical picture. That the injected material had an effect upon the leukemic process was indicated in several ways. The earliest manifestation, perhaps, was a change in the morphologic appearance of the lymphocytic cells from the regular, round, evenly staining forms to very irregular, distorted, and pyknotic cells. This was most apparent in those locations where the leukemic infiltrations were most severe. Although the bone marrow was diffusely involved in all the treated cases, there was a decrease of cellularity to a degree consistent with a diagnosis of hypoplasia. A later result, which suggested healing, was indicated by an increase of reticulum and fibrous tissue with a corresponding decrease of leukemic cells. This was particularly evident in the liver from cases 1 and 7. Finally, the effect of treatment was manifested in the marked pleomorphism evoked especially in the lymph nodes, spleen, and bone marrow, and in the reticulum cell hyperplasia best exemplified in the lymph nodes and spleen.

The injections of organ extracts have caused partial remissions in acute leukemia (Cooke, 1938)<sup>8</sup> but no such change in the pathologic morphology.

#### DISCUSSION

Myelokentric acid is a noncarbinol acid that is found in the urine of patients with acute or chronic myeloid leukemia, chronic lymphoid leukemia, monocytic leukemia, Hodgkin's disease, and in liver lipids. Lymphokentric acid is an hydroxyacid that is found in the urine of patients with acute or chronic lymphoid leukemia, lymphosarcoma, chronic myeloid leukemia, monocytic leukemia, Hodgkin's disease, and in liver lipids. These two acids are chemically interconvertible: i.e., by reduction myelokentric acid may be converted from a noncarbinol to an hydroxyacid and the end product is biologically active as a stimulator of lymphopoiesis, and the reverse is also true: i.e., by oxidation, lymphokentric acid may be converted from an hydroxyacid to a noncarbinol acid and the end product is biologically active as a stimulator of myelopoiesis.

We believe that these substances are of fundamental importance in the abnormal processes of blood cell production in the leukemias. It is also possible that they constitute the balance mechanism in normal blood cell proliferation and maturation. We do not believe that they are the causative agents for leukemia.

Leukemia in the human individual may exist without any known precursor but frequently it occurs following a precipitating incident or set of incidents. These may be exposure to x-ray or radium, contact with or exposure to benzol or its derivatives, trauma—either physical or mental—the use of arsenical or sulfonamide drugs, infection of various types as well as other factors and agents. These incidents or agents are not the causes of leukemia *per se* but each may act to upset the normal balance of blood cell formation so that leukemia results, and it then continues till the death of the individual even if the inciting agent has been removed.

It is our contention that the leukemias represent a group of metabolic disorders in

which the various types are not well separated on a physiologic basis. These metabolic disorders may be expressions of the excesses or deficiencies of myelokentric and lymphokentric acids and/or imbalances in the normal relationship of the lymphoid and myeloid systems.

Ziegler was the first to propose that myeloid and lymphoid cells and tissues were balanced and interrelated in their activities. It has been apparent almost from the earliest classification of blood cells that when the lymphoid system was hypertrophic the myeloid system was diminished in activity and vice versa. Evidence of this type of interrelationship of the two hematopoietic systems occurs in the myeloid response to various bacterial infections and the lymphoid response to certain filter-passing viral infections. It is also evident in certain of the metabolic diseases, as, for example, reduction in the hormones from the cortex of the adrenal is accompanied by myeloid atrophy and lymphoid hypertrophy while in Cushing's syndrome the reverse may be found.

Each of the three large groups of leukemia seem to represent exaggerations of this process. In chronic myeloid leukemia, for instance, hyperactivity of the bone marrow elements occurs. This represents not only an increased production of cells but, along with the increased number of cells, there is also an increase in maturation. Throughout the greater part of the disease there is not only an overabundance of cells but an overabundance of cells that are apparently normal in morphology and biologic reactions. Throughout the greater part of the disease the lymphoid system appears normal and at least normal numbers of lymphocytes appear in the peripheral blood. Toward the end of the disease the lymphoid system is frequently replaced by myeloid cells and the lymphoid cells disappear from the peripheral blood, and at this time, to a large extent, the maturation of the myeloid cells ceases. This is termed the blastic phase of chronic myeloid leukemia and is comparable, if not identical, with the acute form.

We have suggested previously that myelokentric acid and lymphokentric acid are mutually reciprocal in action and that myelokentric acid stimulates myelopoiesis without maturation.<sup>3</sup> We contend that the maturation of myeloid cells is brought about by the action of lymphokentric acid which inhibits the proliferation of myeloid cells and hence allows them to mature. In chronic myeloid leukemia throughout the greater part of the disease there is an excess of myelokentric acid and at least a normal amount of lymphokentric acid. Such a mechanism brings about excessive proliferation and maturation of myeloid cells and normal production of lymphoid cells. As the lymphokentric acid becomes exhausted maturation of myeloid cells ceases, and this coincides with the disappearance of the lymphoid cells from the blood and tissues. The mechanism involved in acute myeloid leukemia is consistent with a lack of lymphokentric acid and a normal or increased value of myelokentric acid. Some acute myeloid leukemias show slight maturation of myelocytes and probably have available small amounts of lymphokentric acid, but in each one there is a deficiency of lymphokentric acid and lymphopoiesis.

A similar picture can be drawn for a chronic lymphoid leukemia with its increased proliferation and maturation. In this disease if the myeloid system becomes

exhausted and the bone marrow is replaced with lymphoid elements less and less maturation of the lymphoid cells will occur and an acute phase will result. This is encountered less frequently than the similar phase of chronic myeloid leukemia.

Again, we have suggested previously that lymphokentric acid brings about lymphoid proliferation without maturation.<sup>3</sup> The maturation of lymphoid cells is brought about by the action of myelokentric acid which inhibits the proliferation of lymphoid cells and hence allows them to mature. In chronic lymphoid leukemia there is an excess of lymphokentric acid and at least a normal amount of myelokentric acid. An excessive proliferation and maturation of lymphoid cells and a normal production of myeloid cells occurs because of such a mechanism. When the myelokentric acid becomes exhausted maturation of lymphoid cells ceases and little or no production of myeloid cells occurs. The mechanism involved in acute lymphoid leukemia is consistent with a lack of myelokentric acid and a normal or increased amount of lymphokentric acid so that lymphopoiesis goes on in a proliferative but unmaturing manner without myelopoiesis.

It is more difficult to explain the monocytic leukemias on this basis. We have reported previously, however, that extracts of the urine from patients with monocytic leukemia contained excessive amounts of both myelokentric acid and lymphokentric acid. Hypothetically the overstimulation of both lymphoid and myeloid systems at the same time might result in a monocytic proliferation accompanied by some pleomorphism and either much or little maturation of the cells involved.

#### SUMMARY

Eight cases of blastic lymphoid leukemia have been treated with myelokentric acid in crude form, because hypothetically in blastic lymphoid leukemia there is a deficiency of this material. The crude myelokentric acid was used because it was more easily obtained than partially purified material. Purification of biologically active materials by methods of extraction and precipitation necessarily results in a considerable loss of material. Thirteen partial remissions occurred following the administration of crude myelokentric acid. Seven of the 8 patients have died, and 5 necropsies were performed.

The necropsy material adds further weight to the belief that the remissions were induced by the myelokentric acid in that in all 5 necropsies there was a definite alteration in the histologic morphology as contrasted with the findings in the necropsies of the controls.

It seems inadvisable, however, to treat a large number of patients with this material because it is crude, it is relatively unavailable, and no standard dose has yet been devised.

We wish to thank Dr. D. L. Turner and Dr. W. A. Hause for valuable assistance in this work.

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# THE BLOOD PLATELETS

## THE RATE OF THEIR UTILIZATION IN THE CAT

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### I. INTRODUCTION

OF ALL the formed elements of the blood the platelet has been the most difficult to study. This has been largely due to its extreme lability in shed blood and the consequent technical difficulties associated with handling it. In particular, there has been little or no valid conception of how long the average platelet may be expected to survive in the intact animal. Estimations of the average rate of platelet utilization have been derived almost entirely from the observed rate of regeneration in animals rendered acutely thrombopenic by one or another means—a method whose validity is open to serious question. In addition to these observations, Duke<sup>2</sup> in 3 patients with thrombopenic purpura was able to make an indirect estimate of platelet life span by noting the duration of improvement in hemostasis following direct transfusion. It is also possible to arrive at some rough estimate of the rate of platelet utilization if one accepts the figures of Howell and Donahue<sup>3</sup> on platelet counts made simultaneously from venous and arterial blood. However, these findings were not confirmed by Fidler and Waters,<sup>4</sup> or by Tocantins and Bradshaw.<sup>12</sup> This brief pertinent literature will be reviewed presently, but one is forced to conclude that there is little in the way of concrete data to support more than a conjecture.

It is the object of this report to present data obtained by *in vivo* studies on the cat relative to the average rate of platelet utilization in that animal. In principle, the method has been as follows. A cat is rendered chronically thrombopenic by means of radiation and then cross circulated via carotid to carotid anastomoses with a normal animal. After equilibrium has been established the thrombopenic animal possesses in the neighborhood of half the platelets originally belonging to the normal cat. Each animal is then returned to its own circulation and the rate of disappearance of the cross circulated platelets is followed by repeated counts. The methods employed will be discussed in detail in a subsequent section. In effect, therefore, the results are analogous to those obtained by following the rate of disappearance of transfused erythrocytes, the radiated animal in this instance having been rendered incapable of producing more than negligible numbers of platelets. Cross circulation serves merely as a device to give the thrombopenic animal a

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massive direct transfusion rich in platelets, the platelets never leaving the vascular system or contacting other than an endothelial surface.

## II. REVIEW OF THE LITERATURE

Only isolated estimates of the *in vivo* rate of utilization of the blood platelet in either animal or man appear in the medical literature. The majority of these reports are referred to by Tocantins<sup>12</sup> in his excellent monograph on the mammalian platelet.

The earliest report of which the authors are aware is that of Duke<sup>2</sup> in 1910. This investigator found that the hemorrhagic diathesis in 3 patients with idiopathic thrombopenia was largely relieved by *direct* blood transfusion. In 1 case the platelet count was reportedly raised from 3,000 to 123,000 platelets per cubic millimeter and in another from 20,000 to 89,000 per cubic millimeter. The transfused platelets had almost completely disappeared within three days. Duke concluded that while these platelets may conceivably have been prematurely destroyed by the disease process, they probably were shortlived bodies whose utilization might be as rapid as one-fourth the total number per day. He also mentioned that experimental results of platelet transfusion in animals rendered thrombopenic by benzol suggested a rapid rate of platelet disappearance. Published details on these experiments could not be found. Duke,<sup>3</sup> again the following year, reported results obtained by observing the rate of platelet regeneration in dogs made thrombopenic by the removal of blood and its reinjection after defibrination. On the average, the rate of regeneration under these circumstances amounted to around one-fifth the entire number in the blood per day. Firket<sup>5</sup> (quoted by Tocantins) obtained essentially similar results in animals rendered thrombopenic by both the defibrination technic and after saponin injections. Similar rates of regeneration have also been noted by Bedson<sup>1</sup> and Tocantins<sup>11</sup> after thrombopenia induced by the injection of antiplatelet serum. In this connection, however, it is also interesting to note that an increase of 400,000 to 1,000,000 platelets per cubic millimeter in twenty-four hours has been observed after splenectomy in patients with thrombopenia.<sup>12</sup>

In 1916 Minot and Lee<sup>8</sup> noted marked improvement in the coagulation time of hemophilic patients after transfusion. This lasted for approximately three days. The authors felt that this was probably due to the introduction of normal platelets and that the clinical results were in corroboration of the findings of Duke. Of course, more recent work has indicated that improvement in hemostasis in hemophiliacs after transfusion is not due to introduced platelets *per se*.

Shause, Warren, and Whipple<sup>10</sup> noted the sudden disappearance of platelets in dogs seven to eight days after they received radiation over the entire bony skeleton. Since megakaryocytes were virtually all destroyed by the amount of radiation given, it was suggested that the life of the platelet in the peripheral blood might be around seven to eight days.

Howell and Donahue<sup>6</sup> in 1937 found arterial blood consistently to contain a larger number of platelets than simultaneously removed samples of venous blood. For this reason, and because of evidence interpreted as indicating that platelet production in abundance took place in the lungs, it was concluded that new platelets

are added to the blood in the capillary areas of the lungs and that a corresponding destruction of platelets occurs as the blood passes through the capillary areas of the systemic circulation. Using the figures given, it is apparent that this would involve a complete replacement of the entire platelet mass in approximately ten complete circulations of the blood. However, Tocantins and Bradshaw<sup>12</sup> (reported by Tocantins) found inconstant relationships between the platelet counts of arterial and venous blood. Fidler and Waters<sup>4</sup> were likewise unable to confirm either a significant differential in the platelet levels of arterial and venous blood or to obtain satisfactory evidence of important platelet production in the lungs.

In view of the conjectural nature of the rate of platelet utilization in either man or animal as suggested by the scanty evidence available in the medical literature, it appeared desirable to investigate the problem by some direct method in which the actual rate of disappearance of normal platelets could be measured.

### III. METHODS

Normal cats were rendered thrombopenic by repeated exposures to known amounts of radiation and then under nembutal anesthesia cross circulated via carotid anastomoses with nonradiated animals. The full details of the method of establishing the cross circulation are given elsewhere.<sup>7</sup> Suffice it to say here that platelets traversing the anastomoses have a continuous endothelial lined pathway in their passage from one animal to the other.

Cross circulation was allowed to function for a sufficiently long time to establish equilibrium and then each animal returned to its own circulation. In eight satisfactory experiments of this nature it was possible to elevate the platelet count of the previously thrombopenic cat anywhere from 100,000 to 450,000 per cubic millimeter. The rate of disappearance of these cross circulated platelets was then followed by repeated counts until they had returned to their pre-cross circulation base line or below.

During the period of developing thrombopenia, which usually took in the neighborhood of ten to twelve days, the platelet count was checked at intervals. No animal was deemed sufficiently thrombopenic for use until the platelet count had fallen to approximately 50,000 per cubic millimeter. In some instances it was much lower. Control animals receiving identical radiation preparation but not cross circulated were repeatedly checked to be certain that no significant platelet regeneration was occurring during the period of the experiment. These animals invariably died with gross purpuric manifestations and extremely low platelet counts during the progress of the experiment or, if they lived for the entire period, showed no evidence of platelet regeneration.

All platelet counts were made with Bureau of Standards certified pipets on blood obtained from the marginal ear vein. Rees-Ecker<sup>9</sup> diluting fluid was first drawn up to the 0.5 mark on a red blood cell pipet. Blood was rapidly drawn up to the 1.0 mark and the pipet then filled in the usual manner with more diluting fluid. All counts were invariably made in duplicate and discarded unless satisfactory checks were obtained. Sufficient numbers of duplicate counts were made each day to preclude the possibility of an occasional erroneous count being given undue signifi-

cance. Results were further checked by examination of Wright stained cover slip preparations for the number of platelets present. Glassware was scrupulously cleaned and any preparations rendered unsatisfactory by contact hemolysis of erythrocytes discarded.

In connection with the cross circulation experiments, observations were made to determine the physiologic variations of platelet counts made by this method on normal cats. In 121 counts made in this manner on 55 normal animals the average platelet count was found to be 422,000 per cubic millimeter. The following percentage distribution was obtained.

<i>Platelet Count</i>	<i>%</i>
100,000-200,000 per c.mm. . . . .	0
200,000-300,000 " " . . . . .	17.4
300,000-400,000 " " . . . . .	32.2
400,000-500,000 " " . . . . .	21.5
500,000-600,000 " " . . . . .	24.0
over 600,000 " " . . . . .	4.9
	<hr/> 100.0

Total dosage of radiation varied somewhat from animal to animal depending on their toleration of the initial exposure and on the satisfactory development of severe thrombopenia. The following factors were kept constant.

Voltage . . . . .	250 K.V.P.
Milliamperage . . . . .	15
Target distance . . . . .	22 inches (to center of cat)
Filter . . . . .	Aluminum parabolic plus $\frac{1}{2}$ mm. copper
Half value layer . . . . .	2.1 mm. copper
Output . . . . .	20-25 r per minute with minor variations

In seven of the eight experiments an initial dose of 250 to 350 r whole body radiation (usually 250 r) was given. One week later the animal received a second dose of 00 r to 300 r (usually 200-250 r). Four to five days after the second exposure a third dose of 100 r to 250 r was given (usually 150 r). Total dosage therefore amounted to 650-800 r over an eleven to twelve day period. Animals in good condition at this time but satisfactorily thrombopenic were used for cross circulation. While some animals did not survive the period of preparation, it was surprising how many animals could be obtained in good condition at the time of operation. In one animal, cat number 64, the radiation preparation was considerably more gradual. This animal was used in the first successful experiment when radiation technic for producing chronic thrombopenia was still in the formative stage.

Since these animals were also markedly granulocytopenic it was customary to administer penicillin in saline subcutaneously at intervals throughout the experiment.

In all but one instance the thrombopenic animal was cross circulated with a normal cat. Cat number 254, however, was deliberately cross circulated with an animal splenectomized some ten days previously. The splenectomized animal in this

case was employed because its high platelet count (over 900,000 per cubic millimeter) made it a particularly desirable donor.

#### IV. PRESENTATION OF DATA

The data are presented in tabular and graphic form.

Table 1 shows the base line platelet level of each animal prior to cross circulation, the level attained immediately after return to independent circulation, and the platelet levels at approximately twenty-four hour intervals for five days thereafter. It is of course impossible to tabulate all the platelet counts performed on each animal. It was customary to perform at least four sets of duplicate platelet counts in the few hours after cross circulation. These showed some fluctuation for a few hours, probably incident to vascular readjustments associated with return to independent circulation and recovery from anesthesia. The first count after cross cir-

TABLE 1.—*Individual and Average Platelet Counts per Cubic Millimeter of Radiated, Thrombopenic Cats before and at Varying Intervals after Cross Circulation with a Normal Cat*

Cat No	Duration of x-circ.	Prior to x-circ.	1st after x-circ.	20-26 hrs. after x-circ.	44-50 hrs. after x-circ.	68-74 hrs. after x-circ.	92-100 hrs. after x-circ.	114-120 hrs. after x-circ.
254	2 hrs. 30 min.	52,000	454,000	264,000	163,000	58,000	21,000	9,000
286	3 hrs. 22 min.	38,000	326,000	246,000	187,000	114,000	67,000	23,000
64	4 hrs. 7 min.	51,000	272,000	129,000	103,000	64,000	53,000*	39,000*
250	3 hrs. 9 min.	13,000	117,000	85,000	36,000	6,000	6,000	6,000
252	2 hrs. 24 min.	26,000	197,000	176,000	119,000	75,000	33,000	7,000
256	2 hrs.	28,000	248,000	153,000	117,000	42,000	9,000	7,000
222	1 hr. 47 min.	15,000	277,000	168,000	119,000	51,000	19,000	12,000
194	2 hrs. 55 min.	44,000	168,000	136,000	51,000*	29,000	8,000	5,000
Total		267,000	2,059,000	1,357,000	895,000	439,000	216,000	108,000
Average		33,375	257,375	169,625	111,875	54,875	27,000	13,500

\* Count made slightly outside the specified time limit.

ulation alone is given for purposes of conserving space. It is reasonably representative. It was also customary to do at least three sets of duplicate counts every day on each animal until the pre-cross circulation base line had again been reached. Again, data are given only on that count closest to twenty-four hours or some multiple thereof after the time cross circulation was discontinued. The counts tabulated are in complete agreement with the rest of the data.

Figure 1 shows graphically the rate of disappearance of cross circulated platelets in four of the eight experiments. Each point represents the average of a set of duplicate platelet counts and the fluctuations mentioned previously can be readily noted. These four experiments were selected for purposes of illustration since the peak platelet level attained varied by approximately equal intervals from around 100,000 per cubic millimeter to better than 400,000 per cubic millimeter.

Table 2 indicates the average rate of platelet utilization per cubic millimeter per hour computed for each animal. It is obvious, in view of some fluctuations in the

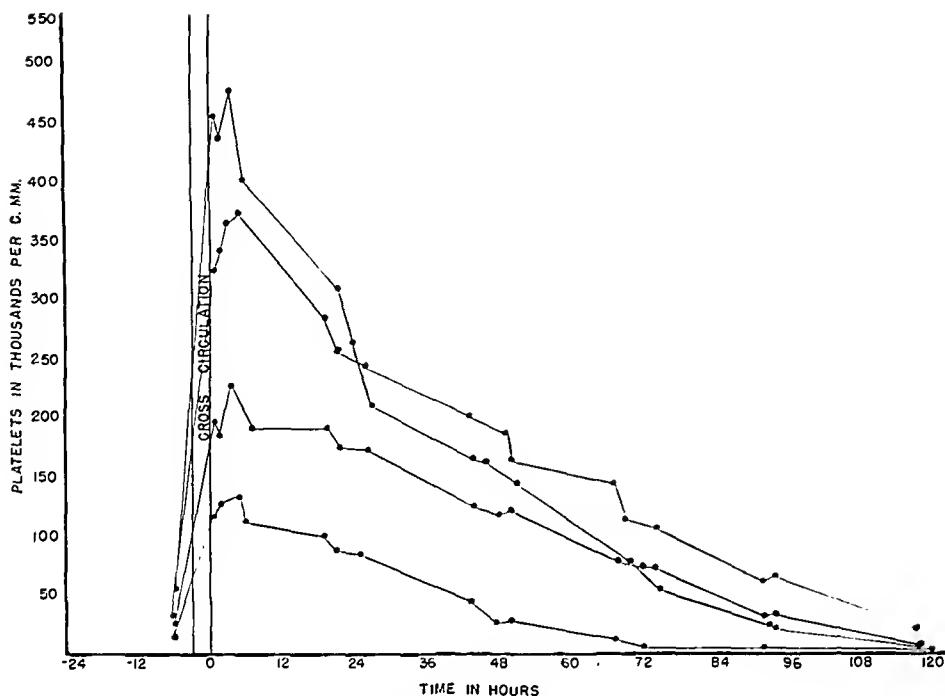


FIG. 1. RATE OF DISAPPEARANCE OF CROSS CIRCULATED PLATELETS IN FOUR OF THE EIGHT EXPERIMENTS. Each point represents the average of duplicate platelet counts made at the indicated time.

TABLE 2.—*Individual and Average Group Rate of Platelet Utilization*

Cat No.	Average Number of Platelets Utilized per c. mm. per hour
254	4,613
286	2,635
64	2,062
250	1,659
252	1,804
256	2,861
222	2,811
194	1,894
Total	20,339
Average for group	= 2,542 platelets utilized per c. mm. per hour.

counts soon after cross circulation and in view of an insufficient number of counts to determine the exact time after cross circulation that the animal returned to its preoperative base line, that the figures given represent an approximation. The platelet level attained after cross circulation was arbitrarily designated as the first count

after return to independent circulation; the time of return to the base line was arbitrarily designated as the time of the first platelet count under 50,000 per cubic millimeter.

#### V. ANALYSIS OF RESULTS

From an examination of table 1 it is apparent that no hard and fast rule can be laid down regarding the rate of platelet utilization which will apply to each animal. This is of course to be expected in the light of the fact that platelets when utilized are almost always destroyed. It would indeed have been surprising had it been possible to note a perfectly uniform rate of fall in one individual, much less uniform rates of disappearance in different individuals. Demands for platelets must of necessity vary rather widely in different animals or in the same animal at different times.

Despite lack of complete uniformity from experiment to experiment, certain important generalities or trends show good correlation. In every instance platelets disappeared from the circulation in a steadily progressive manner with significantly lower counts noted in each successive twenty-four hour period. In many instances, as in three of the four experiments graphed on figure 1, these rates of disappearance roughly paralleled each other, depending on the height of the initial level after cross circulation. Further, there was a general trend for animals receiving relatively slight elevations in platelet count to return to their preoperative base line faster than in those receiving marked elevation in platelet count. The chief exception to this is cat number 254 in which the highest platelet level of the group was obtained. Here, the rate of fall of the cross circulated platelets was relatively precipitous and the average rate of platelet utilization was about twice as great as the average for the rest of the group. This animal alone received platelets from a splenectomized donor. Whether the platelets from this donor had been altered in some manner as a result of splenectomy can only be a matter of speculation.

However, the important feature of the presented data is that it took two to slightly more than four days for cross circulated platelets to disappear from the recipient. Since preliminary observations on normal cats indicated that around 17 per cent had platelet counts between 200,000 and 300,000 per cubic millimeter and 32 per cent had platelet counts between 300,000 and 400,000 per cubic millimeter, the counts obtained in experimental animals were within the physiologic range of about 50 per cent of the normals tested by identical methods. This indicates that under the conditions of this experiment the entire platelet mass in the cat requires replacement every three to five days.

#### VI. DISCUSSION

The data presented constitute the first observations on animals in which rate of platelet utilization has been directly measured. It is somewhat surprising that the results obtained are in rather close accord with estimates made indirectly from observations on the regeneration rate of platelets after experimental thrombopenia.<sup>5,1,11</sup> One would expect the rate of regeneration under the intense stimulus of severe platelet depletion to be a poor index indeed to the normal rate of production

(and hence the normal rate of destruction) in the normal animal. The findings are also in rather close agreement with those of Duke.<sup>2</sup> The data obtained are not, however, consistent with the observations of Howell and Donahue.<sup>6</sup>

A turnover in the entire number of platelets in the body within a period of three to four days is probably a minimal estimate. That is, turnover in the completely normal animal may be slightly slower. The animals employed had received radiation and did have an operative incision to heal. In no case was any infection noted despite close check and in no animal was there apparent loss of blood from any source until marked thrombopenia had recurred. However, it cannot be denied that there may have been some increase in demand for platelets in animals under these conditions. There is no evidence that such an increase in demand would be great if it existed at all.

The sequence of events at the time of operation was dramatic testimonial to the possible role of the platelet in hemostasis. When the neck incision was made in the thrombopenic cat persistent oozing from traumatized vessels was a major problem. Hemostasis was difficult to secure and often oozing persisted to some extent despite all efforts. However, very shortly after cross circulation was established all oozing ceased spontaneously. The field at closure was invariably dry and no difficulty was experienced with bleeding from the wound after operation. It was only three to four days later when the platelet count was again very low that purpuric manifestations recurred. These became progressively worse and were often the cause of death within the following few days. Of course, it is recognized that the platelet *may* have been only one factor in producing this effect on hemostasis. Indeed, it could conceivably have not had any effect at all. The basis for this statement is the observation which has been made repeatedly by us at the time of splenectomy in idiopathic thrombopenic purpura. At the time of splenectomy abnormal oozing of blood frequently stops abruptly when the splenic pedicle is clamped in spite of the fact that the number of platelets in the blood remains unchanged. Thus, the effect on hemostasis in our animal *could* have been due to some other substance or substances in the blood than the platelet. However, the correlation between the bleeding tendency and the platelet level was so close that we feel the platelet must have been at least one important factor with regard to the bleeding.

Care has been taken to refer only to "rate of platelet utilization" rather than to platelet "life span." Since the platelet, unlike the erythrocyte, is destroyed when used, data on rate of disappearance of transfused platelets are a clue mainly to platelet demands. They disclose nothing as to how long a platelet might survive without spontaneously disintegrating if there were no demands for its use.

In the light of the experiments recorded here, the observations of Duke<sup>2</sup> in human beings deserve some further comment. If they could be substantiated, massive direct transfusion would certainly be of importance in tiding patients with idiopathic thrombopenic purpura over critical periods of bleeding or in preparing them for splenectomy. Significant improvement in hemostasis might warrant the use of the more inconvenient direct transfusion rather than the customary method of transfusing stored blood in which platelets are rapidly destroyed. In the experiments reported by Duke, neither the exact amount of blood transfused nor the

details of the direct method employed are indicated. In one of the experiments the platelet level was raised approximately 120,000 per cubic millimeter (3,000-123,000). If the donor blood contained a normal number of platelets, one would have to assume that the recipient was given approximately 2500 cc. of blood. Whether such large quantities of blood were given cannot be determined from his data. However, we have recently given a direct transfusion of 2000 cc. within twenty-four hours to a severely thrombopenic patient with aplastic anemia (platelet count below 10,000 per cubic millimeter). The multiple syringe method was used and 1500 cc. of the transfused blood given within a three hour period. The other 500 cc. had been given about fifteen hours previously. The platelet count was never detectably raised and no improvement in purpuric manifestations occurred. Bleeding from the nose, present before transfusion, continued unabated afterwards. The failure to obtain any elevation of platelet count or improvement in hemostasis is unexplained. Experiments are contemplated to determine if possible what happens to the blood platelet during its brief sojourn outside the body during a direct transfusion. It should be emphasized, however, that direct transfusion methods currently in use are not comparable to the continuous endothelial anastomoses of the animal experiments. One experiment, of course, proves nothing, and more trials of a similar nature employing other types of direct transfusion apparatus will have to be made. Experiments of this type are being carried out as suitable patients appear in the clinic. Careful observations on a few patients should give a definite answer as to whether the platelet level of the human being can be raised significantly with a corresponding beneficial effect on hemostasis in patients with thrombopenia by means of direct transfusion of blood. If such should prove to be the case, direct rather than indirect transfusion should be used in thrombopenic individuals.

#### VII. SUMMARY

1. The rate of utilization of blood platelets in radiated, thrombopenic cats has been measured directly. Thrombopenic animals, incapable of significant platelet regeneration, were cross circulated via carotid to carotid anastomoses with normal animals. After return to independent circulation the rate of disappearance of cross circulated platelets was measured by periodic counts.

2. By this method it was possible to elevate the platelet count anywhere from 100,000 to around 400,000 per cubic millimeter. The highest count obtained followed cross circulation with a splenectomized animal. In most instances the platelet level attained was within the physiologic range of that found for normal cats by the same method.

3. The cross circulated platelets gradually disappeared from the circulation over a two to a slightly more than four day period. Under the conditions of this experiment the entire platelet mass would have to be replaced therefore every two to five days. The same figures probably apply within narrow limits to the normal cat.

4. The average rate of platelet utilization was approximately 2500 per cubic millimeter per hour. In seven of eight experiments the rate of disappearance varied

from about 1600 per cubic millimeter per hour to about 2800 per cubic millimeter per hour. In the experiment using a splenectomized donor the rate of disappearance was about double the average for the rest of the group.

5. Attention is called to possible therapeutic implications of these findings in idiopathic thrombopenic purpura.

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## FOLIC ACID IN THE TREATMENT OF PERNICIOUS ANEMIA

By LEO M. MEYER, M.D.

BEGINNING in 1941 a group of investigators demonstrated that there is present in liver, yeast, spinach, and grasses a substance which is necessary for the growth of the *Lactobacillus casei* and the *Streptococcus faecalis* R.<sup>1-6</sup> This substance, which has been known as the *L. casei* factor, or folic acid, was isolated and crystallized in 1943 and synthesized two years later.<sup>7,8</sup> The structural formula was announced as pteroylglutamic acid in 1946.<sup>9</sup> During this period another group of investigators showed that the hematological changes (anemia and leukopenia) which developed in rats sustained on a synthetic diet plus various sulfonamide compounds could be prevented or cured if the animals received either crude or synthetic *L. casei* factor.<sup>10-17</sup> It was similarly found that the anemia, leukopenia, diarrhea, and oral lesions which developed in monkeys fed a vitamin M-deficient diet could be entirely relieved by the administration of folic acid.<sup>18</sup>

Experiments with folic acid produced highly suggestive results in the treatment of the various macrocytic anemias. In 1944 Castle and his co-workers fed purified casein with various accessory factors of the vitamin B group to patients with pernicious anemia.<sup>19</sup> They included folic acid among the substances tested but obtained no hematological or clinical responses. However, the quantity of folic acid used was only 2.3 to 3.6 mg. daily. A series of papers by Spies and his co-workers reported the results of treatment with folic acid in pernicious anemia, nutritional macrocytic anemia, and sprue.<sup>20-24</sup> These authors noted clinical improvement on the 3rd to the 5th day, which preceded the reticulocyte rises. The appetite became increased and glossitis gradually diminished. Paresthesias were also improved but did not entirely disappear. This was the only reference made to involvement of the nervous system. The hemoglobin and erythrocyte levels did not reach normal and the reticulocyte responses were not as high as might have been anticipated with liver therapy. Moore and his group described their experiences with 2 cases of pernicious anemia, 1 of sprue and 1 of macrocytic anemia of pregnancy treated with folic acid.<sup>25</sup> All of the patients improved clinically, had satisfactory reticulocyte responses, and showed increases in the hemoglobin and red cells. In the course of the 3 to 5 weeks during which the patients were followed, completely normal figures were not obtained. Darby and Jones also used folic acid in the treatment of sprue with similar clinical and hematologic results.<sup>26,27</sup> One of their patients, however, reached a level of 5.0 million red cells. Zuelzer and Ogden described a megaloblastic anemia in infants and children in which a series of 8 cases was treated with folic acid.<sup>28</sup> Here again normal blood levels were not reached during the period of time covered in the report.

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The present investigation was undertaken to study the effect of prolonged treatment of pernicious anemia with folic acid on the blood picture and neurological symptoms. Another group of patients was treated with suboptimal doses of liver extract ( $\frac{1}{2}$  unit) plus folic acid to determine whether this combination would produce a complete hematological remission and protect against or improve nervous system disease. One patient with macrocytic anemia following extensive resection of the small and large intestines is included since his clinical picture presented a sprue-like syndrome.

*Case 1.*—E. W., white female, aged 80, was admitted to the Kings County Hospital because of weakness and fecal and urinary incontinence of 4 months' duration. The patient was disoriented and orthopaedic. Appetite was poor. On examination her tongue was bright red and smooth. Slight icterus was noted. A decubitus ulcer was present over the sacrum. Premature ventricular contractions were present. Neurological examination was unsatisfactory but knee and ankle jerks were absent.

Laboratory data: No free HCl was found in gastric juice after histamine. Gastrointestinal series was negative. The stools contained no occult blood. The Wassermann test was negative. Urine contained numerous clumps of white blood cells. Hb. and red cells were 8.0 Gm. and 2.2 million. Bone marrow contained 120,000 nucleated cells, 77 megakaryocytes, and 6 per cent megaloblasts. Other laboratory data were blood sugar 286 mg./100 cc.; icterus index 5; cephalin flocculation 2 plus; total protein 5.6 Gm./100 cc.; hematocrit 22.0 per cent. The patient was placed on an oral daily dose of 50 mg. of folic acid. It was difficult to get the patient to swallow the tablets or any food. By the end of the first week, however, she voluntarily asked for food and accepted medication peacefully. The mental status did not improve, however, and it was felt that this resulted largely from cerebral arteriosclerosis. The glossitis gradually improved and finally showed a complete response. Reticulocytes rose to a maximum of 7.8 per cent as compared to an anticipated rise of 14 per cent with liver extract. The hemoglobin and red blood cell levels increased to 12.0 Gm. and 4.35 million respectively. The patient died of bronchopneumonia on the 38th day, so that an extensive hematologic follow-up could not be made.

Comment: This patient showed a submaximal reticulocytosis but a satisfactory rise in hemoglobin and red blood cells. No conclusion can be reached regarding complete hematological remission since the patient expired on the 38th day of treatment. However, the clinical improvement and return of appetite and general well-being are similar to those noted by other investigators.

*Case 2.*—S. B., white male, aged 56, was admitted to the Kings County Hospital because of weakness, pallor, and inability to walk for the past 6 months. The patient had been told he had pernicious anemia  $\frac{1}{2}$  years previously but had had no therapy for the last half year. His appetite was poor. Paresthesias of the hands were present. Examination revealed 2 plus edema of the legs; a tongue which was smooth at the tip; hyperactive knee jerks with ankle jerks absent on the left side. Position sense was intact. Vibratory sense was absent at ankles, knees, and iliac crests.

Laboratory data: No free HCl was found in the gastric juice after histamine. Gastrointestinal series was negative except for some diverticula in the sigmoid. The Wassermann test was negative. Other data were icterus index 11.0; cephalin flocculation 2 plus; total protein 5.9 Gm./100 cc.; hematocrit 2.0 per cent. The blood counts are listed in figure 1. Bone marrow contained 70,000 nucleated cells, of which 11.0 per cent were megaloblasts. The patient was placed on a regular ward diet and given 50 mg. of folic acid by mouth daily. Within 72 hours he experienced a striking increase in appetite, so that by the 5th day he was eating double portions at each meal. On the 20th day the paresthesias were diminished. A neurological examination on the 25th day disclosed negative Romberg; moderately ataxic gait; normal position of toes; and absent vibratory sense at ankles, knees, and crests. On the 59th day the dosage was changed to 25 mg. a day. The reticulocytes reached a peak of 37.3 per cent on the 8th day, as compared with an anticipated rise to 39 per cent with liver extract. During this period the Hb. and red cells had reached a stationary level of about 11.0 to 12.0 Gm. and 4.25 million respectively. Several experimental actions of liver were given on the 79th, 85th, 91st, and 98th days, but no distinct improvement in the blood picture was noted. At the latter time a neurological examination disclosed a markedly positive

Romberg test, severe ataxia, position sense of right toe impaired, and absence of vibratory sense up to lower end of sternum. On the 109th day the patient was started on parenteral liver extract and oral yeast therapy. When seen on the 150th day the Hb. was 14.0 Gm., R.B.C. 5.00 million. The patient was walking better and had fewer paresthesias of the hands. The position sense was normal in both toes and the Romberg test was equivocal.

Comment: This patient showed a satisfactory reticulocyte response but a plateau of the hemoglobin and red blood cell counts at somewhat less than normal values was reached. The clinical response was dramatic and encouraging. However, the gradual increase in severity of the neurological signs and symptoms became very pronounced after 3 months and the therapy was changed from oral folic acid therapy to the parenteral administration of liver extract. This resulted in a rise of the hemoglobin and red blood cells to normal levels and a distinct improvement in symptoms and signs of subacute combined sclerosis.

Case 3.—S. M., white male, aged 69, was admitted to the Kings County Hospital because of swelling of right side of neck with choking sensation of one year's duration. The patient had had mild diabetes during the past 3 years, during which time he had lost 15 pounds. A sore tongue was noted 2 months before admission, together with mild anorexia and paresthesias of the left hand. On physical examina

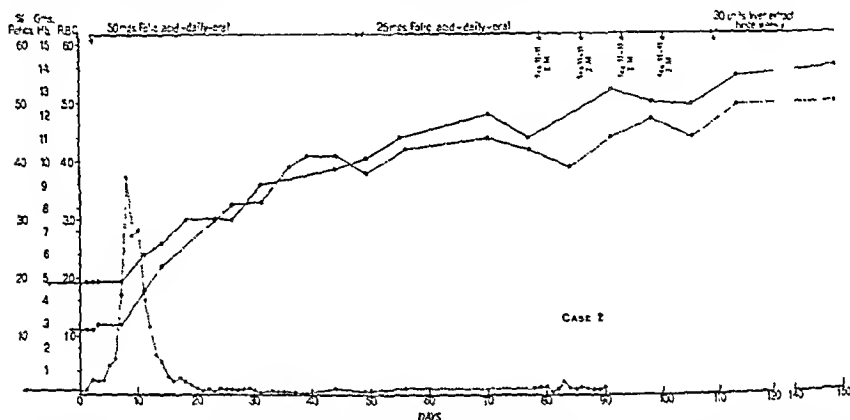


FIG. 1. Case 2. The effects in pernicious anemia on hemoglobin, red cell counts, and reticulocytes following the administration of folic acid 50 mg. orally daily. There is a striking and abrupt reticulocytosis and a sustained but rather slow rise in red cell count. The red count reaches normal figures after liver extract injections are given.

tion a mass 10 by 6 cm. in diameter was noted above the right clavicle. The tongue was not smooth. No signs of thyroid toxicity were present. The prostate gland was moderately enlarged. Vibratory sense was absent at the ankles and diminished at the knees.

Laboratory data: No free HCl was present in the gastric juice after histamine. Stools were negative for blood. Gastrointestinal series disclosed no ulceration or neoplasm. The trachea was shifted to the left. Hypertrophic arthritis of the 1st and 2nd lumbar vertebrae and of the left hip joint was found. Other data were icterus index 24; cephalin flocculation 1 plus; blood sugar 174 mg.; creatinine 1.0 mg.; urea 42 mg.; cholesterol 119 mg.; total protein 5.8 Gm.; Wassermann negative. Sternal puncture showed a megaloblastic bone marrow. Blood counts are listed in figure 3. The patient was placed on a regular ward diet and given 20 mg. of folic acid intramuscularly, daily. Within 5 days a noticeable clinical improvement was evident. Appetite increased and a pink color appeared in the patient's cheeks and lips. On the 14th day he reported fewer paresthesias of his hands. Twenty-six days after therapy was begun the patient noted slight vibratory sensation at the left ankle. The blood picture and reticulocytes increased as indicated in figure 2. The peak reticulocyte count was 17.1 per cent as compared with an anticipated height of 22 per cent with liver extract. However, the Hb. and erythrocytes reached a plateau of 12.0 Gm. and 4.2 to 4.5 million, and on the 85th day the patient began to complain of paresthesias of

his hands. In the meantime Feosol (6 tablets per day) had been started on the 72nd day, and the Hb. and red cells rose to a height of 13.7 Gm. and 6.0 million respectively. Despite this hematological improvement, on the 98th day the patient complained of increasing paresthesias of the hands and feet. The vibratory sense was now lost in the right lower extremity up to and including the iliac crest and was greatly diminished on the left. No other neurological findings were present. At this time it was decided to start liver extract therapy (30 units twice weekly) and folic acid was discontinued. This resulted in a rise of Hb. and red cells so that on the 147th day they reached 15.0 Gm. and 6.3 million respectively. The paresthesias of the feet disappeared, the hands improved, and on the last date indicated the vibratory sense was normally present in both lower extremities.

Comment: This patient presents a somewhat similar picture to Case 2. The reticulocyte peak was slightly under the anticipated level and the hemoglobin and red blood cells reached a plateau below the desirable figures. The administration of oral iron therapy in addition to the intramuscular folic acid resulted in a satisfactory hematological remission but the downward progression of neurological signs and symptoms continued. When folic acid was discontinued and intramuscular injections of liver extract

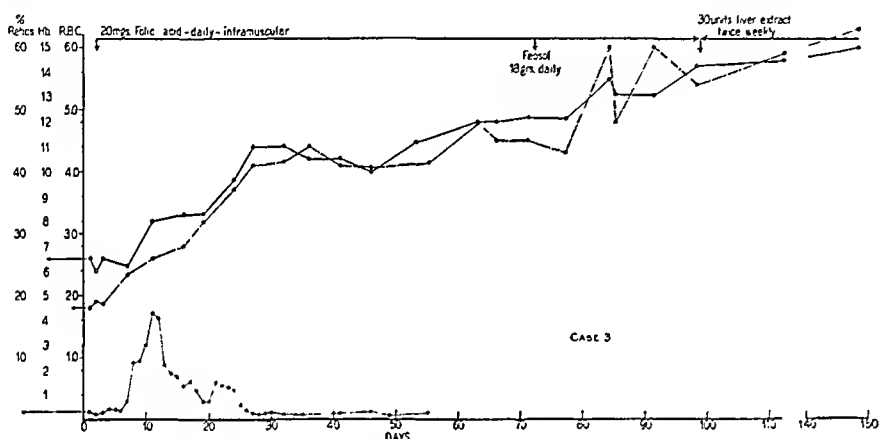


FIG. 2. Case 3. The effects in pernicious anemia on hemoglobin, red cell count, and reticulocytes following the administration of folic acid 20 mg. daily intramuscularly. There is a sustained reticulocytosis followed by a relatively slow increase in red cell count. The hemoglobin level was appreciably improved by the administration of ferrous sulfate orally.

were substituted nervous system changes gradually disappeared and the patient was almost entirely free of symptoms. The blood picture continued well above normal levels.

Case 4.—W. M., adult white male, aged 62 years, was admitted to the New York Hospital because of weakness and dizziness of 6 months' duration. Associated symptoms were a sore tongue, dyspnea on exertion, and palpitation of the heart. There was also numbness and tingling of the feet with cramps in the leg muscles. On examination the liver was felt 1 cm. below the costal margin; the tip of the spleen was barely felt. The positive neurological findings were hyperactive ankle and knee jerks; right ankle clonus; diminished vibratory sensation in both lower extremities, more so on the left.

Laboratory data. No free HCl present in gastric juice after histamine; stools negative for occult blood; Mazzini test negative; gastrointestinal series and barium enema normal; urea nitrogen 23.0 mg.; total protein 7.9 Gm.; icterus index 8; calcium 9.3 mg.; lumbar puncture negative. The bone marrow was megaloblastic. Initial blood count was Hb. 6.0 Gm., red cells 1.9 million. The patient was given a regular ward diet plus 25 mg. of folic acid daily, by mouth. There was a rapid diminution in the patient's complaints with an improvement in his general well-being. After 52 days of treatment the only positive neurological signs were diminution of the vibratory sense at both ankles and knees with absence of knee jerks. The patient still complained of feeling tired. The maximal reticulocyte count was 12.5 per

cent on the 6th day, as compared with an anticipated rise to 20 per cent with liver extract. When seen on the 129th day of treatment the blood count showed a level at about 12.5 to 13.0 Gm. of hemoglobin and 4.0 to 4.3 million red cells. The patient, at this time, complained of severe ringing in the ears and of being dizzy, tired, and weak for the past 3 weeks. Neurological examination on this day disclosed complete absence of vibratory sense at the ankles but present at the knees. The knee jerks were extremely hyperactive.

Comment: The patient showed a relatively poor reticulocyte response to oral folic acid with a satisfactory clinical improvement. The hemoglobin and red blood cells remained below the normal levels up to 129 days of treatment, and a gradual increase in signs and symptoms of nervous system disease developed. The patient has been placed on intramuscular liver extract therapy but sufficient time has not elapsed to evaluate the efficacy of this change in treatment.

*Case 5.*—J. W., adult white male, aged 58 years, was admitted to the New York Hospital because of progressive weakness of 4 years' duration. For the first 3 years of his illness the patient had received liver therapy, to which he had responded well. The positive physical findings were the presence of a recent hemorrhage into the right fundus associated with tortuous retinal vessels. The liver edge was felt 4 cm. below the right costal margin and the spleen 3 cm. below the left costal margin. The only neurological finding was a slight hyperreflexia.

Laboratory data: Mazzini test negative; urea nitrogen 35 mg.; total serum protein 7.3 Gm.; icterus index 11; stools negative for blood; free HCl absent in gastric juice after histamine; hypotonic saline test for erythrocyte fragility normal; gastrointestinal series and barium enema were negative. Bone marrow aspiration disclosed 5 per cent megaloblasts and 29 per cent erythroblasts. Hb. and red cells were 3.8 Gm. and 1.8 million, respectively. The patient was given a regular ward diet and 50 mg. of folic acid daily, orally. There was gradual improvement with disappearance of the weakness and fatigue. The peak reticulocytosis was 26.6 per cent on the 10th day, as compared with an anticipated rise to 40 per cent with liver extract. After 12 days of folic acid therapy a bone marrow aspiration showed 1 per cent megaloblasts and 3 per cent erythroblasts. The reticulocytes fell to the pretreatment level on the 26th day and the following day the folic acid was discontinued and liver extract, 15 units intramuscularly daily, was begun. The blood picture continued to improve and 5 months after the beginning of folic acid therapy, and 4 months after liver therapy, the hemoglobin and red cells were 13.9 Gm. and 5.5 million respectively.

Comment: This patient showed clinical and hemarologic improvement on oral folic acid therapy. The reticulocyte response, however, was below that which would have been obtained with liver therapy. An attempt to produce a secondary response with liver extract after the reticulocytes returned to pretreatment levels was unsuccessful. It is significant that folic acid resulted in changes in the bone marrow from a megaloblastic to a normoblastic type of erythropoiesis similar to that observed with liver extract.

*Case 6.*—T. F., adult white female, aged 60, was admitted to the New York Hospital because of weakness and fatigue of 15 months' duration. At the onset of her illness the patient noticed soreness and burning of her tongue, mouth, and throat, and an associated tingling sensation in the fingers and toes, with loss of taste and smell. Recently the patient complained of dyspnea and palpitation on exertion and a weight loss of 15 pounds. Physical examination showed the tongue smooth at the edges and the spleen palpable 2 cm. below the costal margin. The neurological status was entirely negative.

Laboratory data: Mazzini test negative; no free HCl in gastric juice after histamine; stools negative for blood; gastrointestinal series normal; urea nitrogen 19 mg.; protein 6.3 Gm.; serum bilirubin 1.1 mg.; hematocrit 13 per cent. Hb. and red cells were 4.8 Gm. and 1.5 million. Bone marrow aspiration showed 5 per cent megaloblasts and 20 per cent macro-erythroblasts. The patient was placed on a regular ward diet and given 15 mg. of folic acid, orally. On this regimen there was a distinct improvement in the patient's clinical condition. On the 16th day of treatment taste sensation returned and the tingling sensations in the extremities disappeared. The reticulocytes rose to a peak of 14.4 per cent on the 5th day, as compared with an anticipated level of 31 per cent with liver. On the 20th day the folic acid was discontinued and parenteral liver therapy (15 units daily) was instituted. This produced no secondary reticulocytosis and the Hb. and red cells continued to rise slowly.

**Comment:** This patient presents a similar picture to patient 5. The patient responded well to smaller doses of folic acid orally with improvement clinically and hematologically. However, the reticulocyte response was less than half of what might have been anticipated with liver extract. With the institution of parenteral liver extract therapy no secondary reticulocytosis occurred.

**Case 7.**—B. K., adult white male, aged 47, was admitted to the Kings County Hospital because of weakness of  $1\frac{1}{2}$  years' duration. Occasional dizzy spells were present. No gastrointestinal or neurological symptoms were elicited. Physical examination including a study of the nervous system was entirely negative.

**Laboratory data:** No free HCl was present in the gastric juice after histamine. No blood was found in the stools. Gastrointestinal series was negative. Other data were icterus index 22; urea 29 mg.; cephalin flocculation 1 plus; Wassermann test negative. Bone marrow was megaloblastic. Hb. and red cells were 9 Gm. and 2.6 million, respectively. The patient was given a regular ward diet and treated with 10 mg. of folic acid and  $\frac{1}{2}$  unit of liver extract intramuscularly, daily. On this regimen rapid clinical improvement became apparent. The appetite returned to normal in 2 days and the patient began to gain some weight. His color improved and the blood counts rose as indicated. On the 41st day of treatment the Hb. and red cells were at normal levels and these continued to rise and have remained between 15.0 to 16.0 Gm. and 5.0 to 6.0 million respectively. The reticulocytes reached a peak of 25.0 per cent on the 11th day as compared to an anticipated rise of 11 per cent with liver extract. On the 80th day of treatment the patient was placed on 30 mg. of folic acid twice weekly, intramuscularly. No change was noted in the blood picture, and on the 112th day the dosage was changed to 15 mg. biweekly. During the entire period and up to the 119th day of observation, the patient at no time showed any evidence of nervous system involvement.

**Comment:** This patient was given the combination of suboptimal doses of liver extract with folic acid, and as reported, the clinical response matched that noted when folic acid alone was administered. More significant, however, were the reticulocytes, which doubled the anticipated response, and the Hb. and red cells, which reached normal figures in 41 days. It would appear that the addition of folic acid to liver extract produces a greater reticulocytosis with a normal blood count sooner than when only liver extract is used.

**Case 8.**—M. L., white male, aged 87, was admitted to the Kings County Hospital because of weakness and poor appetite of 1 year's duration. For the 3 months prior to admission epigastric distress had been noted. There were no other symptoms referable to the gastrointestinal or neurological systems. On physical examination the positive findings were: tongue smooth at the edges; liver palpable 2 cm. below the costal margin; enlarged prostate and external hemorrhoids.

**Laboratory data:** Urine contained numerous pus cells; no free HCl in gastric contents after histamine; stools contained blood (small hemorrhoids present); gastrointestinal series negative except for diverticula in the descending colon; icterus index 5; blood sugar 98 mg.; N.P.N. 35 mg.; cephalin flocculation 3 plus; total protein 7.3 Gm. The bone marrow was megaloblastic. Hb. and red cells were 5.9 Gm. and 1.7 million. The patient was placed on the regular ward diet and given 5 mg. of folic acid and  $\frac{1}{2}$  unit of liver extract, intramuscularly, daily. Clinical improvement in the patient's condition was noted after 5 days, followed by a slow but distinct recovery. There was gradual appearance of color in the cheeks and lips with a return of appetite and normal bowel habits. He was able to get out of bed on the 14th day and, in spite of his age, reported that he felt well. The reticulocytes rose to 16.4 per cent on the 7th day, as compared with an anticipated rise to 26 per cent with liver extract. Although the peak was not as high as anticipated there was a plateau between 10 per cent and 16 per cent for 6 days. The hemoglobin and red cells rose slowly and reached a stationary level of about 12.0 Gm. and 4.2 million respectively on the 54th day. No evidence of progression of neurological signs appeared but the urine still contained large numbers of pus cells.

**Comment:** This patient represented a severe test of efficacy of this treatment, because of his age, arteriosclerosis, enlarged prostate, cystitis, hemorrhoids, and diverticula. While the reticulocytes did not rise to the desired peak it was felt that the elevated plateau of 10 per cent to 16 per cent for 6 days compared favorably with the more usual quick rise and fall. The stationary level of Hb. and red cells

below normal figures was probably due to the above-mentioned complications which are known to prevent complete hematological remission even with intensive liver therapy.

**Case 9.**—A. P., white female, aged 49 years, was admitted to the Kings County Hospital because of weakness of 4 years' duration. No history of glossitis was obtained. The only gastrointestinal symptom was an occasional loose tarry stool. The patient was not mentally clear, often contradicting herself, and gave a disconnected story. The only positive physical findings were smoothness of the tongue at the edges and a diminution of position sense in the left big toe.

Laboratory data: Cephalin flocculation 1 plus; icterus index 10; urea 42 mg.; sugar 106 mg.; blood Wassermann negative; cholesterol 210 mg.; no free HCl in gastric juice after histamine; stools negative for blood. X-ray studies of chest and gastrointestinal tract were negative. Bone marrow puncture was refused by the patient. Blood studies are listed in figure 3. The patient was placed on the regular ward diet and treated with 10 mg. of folic acid and  $\frac{1}{2}$  unit of liver extract, intramuscularly, daily. Clinical response with a remarkable improvement in the patient's well-being was apparent within 72 hours

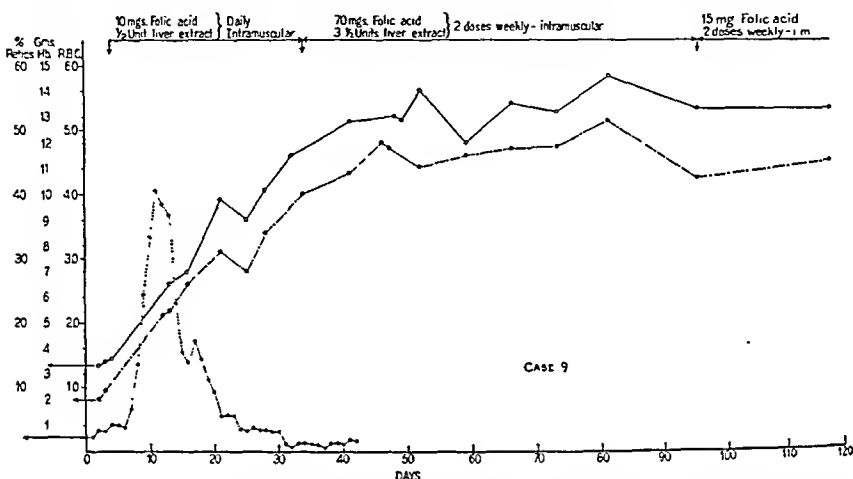


FIG. 3. *Case 9.* The effects in pernicious anemia on hemoglobin, red cell count, and reticulocytes following the administration of folic acid 10 mg. orally daily combined with liver extract  $\frac{1}{2}$  unit intramuscularly daily. Note the sharp and marked increase in reticulocytes which is followed by an unusually satisfactory red cell increase.

The return in appetite was exceptional, and she complained constantly of not getting enough to eat. On the 21st day there was a change of personality in the patient and her stream of thought improved. Except for a tendency to frequent upper respiratory infections the convalescence was uneventful. The peak reticulocytosis was 40.5 per cent on the 7th day as compared with an anticipated rise to 44 per cent with liver extract. The Hb. and R.B.C. rose rapidly and reached normal levels on the 66th day. At this time the patient felt entirely well. The tongue was normal and no neurological signs or symptoms were present after 117 days of treatment.

**Comment:** This patient showed the complete efficacy of treatment using combinations of small doses of liver extract and folic acid. The clinical response was excellent; the reticulocyte response satisfactory; and the hematological remission complete. In addition, the minimal signs of glossitis and nervous system involvement were entirely relieved.

**Case 10.**—C. F., white female, aged 60, was admitted to the Kings County Hospital because of a burning sensation in her throat and abdomen of 1 year's duration. The patient complained of mild anorexia,

weakness, "indigestion," and constipation. She had lost 20 to 30 pounds in the 6 months prior to admission to the hospital. Physical examination showed slight icterus and her liver was felt 2 cm. below the right costal border. Positive neurological signs were an equivocal Romberg and slight impairment of vibratory sense at both ankles.

Laboratory data: Total protein 6.6 Gm.; total cholesterol 155 mg.; blood sugar 82 mg.; urea 44 mg.; cephalin flocculation 4 plus; icterus index 20; stools negative for occult blood. Gastric analysis showed absence of free HCl after histamine. X-ray studies of gastrointestinal tract were negative. The bone marrow was megaloblastic. Blood counts are presented in figure 4. The patient was placed on a regular ward diet and treated with 5 mg. of folic acid and  $\frac{1}{2}$  unit of liver extract, intramuscularly, daily. A subjective clinical improvement was apparent in 48 hours. Her appetite increased and a pink color appeared in her lips and cheeks. The patient complained less of the burning taste in her mouth, throat, and abdomen. The "indigestion" and weakness disappeared. On this regimen the reticulocyte peak was 41.3 per cent on the 9th day, as compared with an anticipated rise to 25 per cent with liver extract. The Hb.

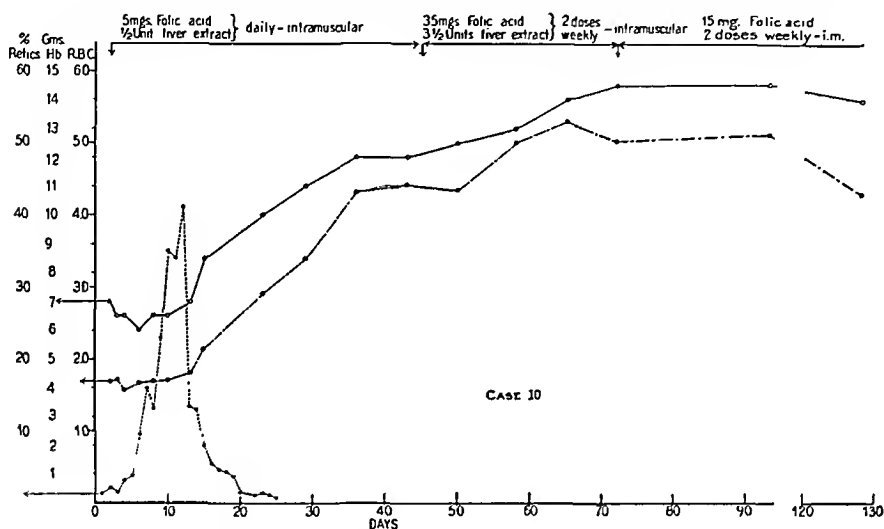


FIG. 4. Case 10. The effects in pernicious anemia on hemoglobin, red cell count, and reticulocytes following the administration of folic acid 5 mg. orally daily combined with liver extract  $\frac{1}{2}$  unit intramuscularly daily. Note the abrupt and striking response in reticulocytes followed by an unusually marked red cell increase.

and red cells reached a normal level of 13.0 Gm. and 5.3 million on the 65th day and have not fallen since. The neurological status was entirely negative 100 days after treatment was started.

Comment: This patient also showed an excellent response to treatment with folic acid combined with small doses of liver extract. The clinical response was completely satisfactory and the reticulocytes rose to almost twice the anticipated figures; the hematological remission was complete. The minimal neurological signs and symptoms were entirely dissipated and the gastrointestinal complaints were reduced.

Case 11.—J. A., white female, aged 30, was admitted to the Kings County Hospital because of progressive weakness, and numbness and spasticity of the legs of 3 months' duration. The patient was unable to walk alone or to control her bowels and bladder. She had had amenorrhea for 4 months and had lost 5 pounds in the last 4 weeks. A history of a severe sore red tongue 1 year previously was elicited. Physical examination disclosed no atrophy of the lingual papillae. Vibratory sense was absent in both lower extremities and up to the level of the 5th lumbar vertebra. Position sense in lower extremities was gone. Knee and ankle jerks and biceps reflexes were hyperactive. Gait was ataxic and bilateral clonus and a positive Babinski response were present.

Laboratory data: Urea 35 mg.; blood sugar 88 mg.; icterus index 12; blood and spinal fluid Wassermann negative; no blood found in stools. No free HCl was present in the gastric juice after histamine. Gastrointestinal series was negative. The bone marrow was megaloblastic. Hb. and red cells were 9.0 Gm. and 2.9 million. The patient was placed on a regular ward diet and injected with 10 mg. of folic acid and  $\frac{1}{2}$  unit of liver extract, intramuscularly, daily. A dramatic improvement followed. In 48 hours her appetite was improved. Three days later the patient felt "rested" in her legs and the "tightness" was gone. Within 5 days her appetite was so ravenous that she ate a double trayful of food at each meal. Sensations of "shocks" in her legs disappeared on the 7th day. Thirteen days after the beginning of treatment the patient menstruated for the first time in 4 months. She began to walk, holding on to the bed or chairs, on the 17th day. Control of bowels and bladder was regained on the 22nd day. On the 25th day her legs felt stronger and a neurological examination on the 33rd day disclosed no changes in the organic reflexes as compared to those on admission. The patient continued to improve clinically but the difficulty in walking remained. The peak reticulocytosis was 25 per cent on the 13th day. However, the patient had a prolonged elevation of reticulocytes from the 7th to the 21st day. The anticipated reticulocyte average with liver extract would have been 8 per cent. The maximal blood counts reached were Hb. 12.6 Gm. and 4.75 million red cells. On the 57th day the patient was started on 30 units of liver extract, intramuscularly, three times a week, plus vitamin B complex and brewer's yeast. On the 98th day the Hb. reached 12.9 Gm. and the R.B.C. 4.7 million. The objective neurological signs were unchanged.

Comment: This patient represented the most dramatic case in the group studied. The clinical improvement with relief of neurological symptoms was so dramatic that it was felt justifiable to continue this form of therapy to evaluate its efficacy. As described, all of her complaints referable to gastrointestinal, neurological, endocrine, and urological systems were relieved to an unexpected degree. The reticulocyte response was triple that anticipated with liver extract and hematological remission was almost complete. It was felt, however, because of the severity of the nervous system involvement and the youth of the patient, that more intensive antineuritic therapy was warranted and so folic acid was discontinued and liver, vitamin B complex, and brewer's yeast substituted. After 40 additional days of this therapy the Hb. and red cells were still at the same levels. Neurological examination was essentially the same.

Case 12.—A. L., white male, developed nausea, vomiting, diarrhea, and weight loss at the age of 16 in 1926. A macrocytic anemia was found. The diagnosis at the time was ulcerative colitis. The patient was given oral liver therapy and clinical and hematological improvement followed. Two years later he developed a tuberculous pleural effusion and was treated at a sanatorium. Following this he suffered numerous attacks of diarrhea, anemia, and weight loss which responded moderately well to diet. In 1939, at the age of 29, he showed signs of intestinal obstruction. The terminal ileum and ascending colon were resected for regional ileitis. The following year macrocytic anemia was discovered and he was successfully treated with intramuscular injections of liver extract at the New York Hospital. After 6 months of therapy he stopped treatment for 4 months. When he returned to the clinic he developed severe urticaria after each injection of liver extract. Various products, including lamb liver extract, were tried, but he reacted violently with urticaria and occasionally with vasomotor collapse. Oral therapy with ventriculo and yeast was of no avail. He was maintained during the following 4 years with frequent transfusions but these had to be discontinued because of unfavorable reactions. In November 1945 the patient's blood count showed hemoglobin 5.8 Gm. and red cells 2.3 million. Folic acid 50 mg. daily, orally, was begun. The patient had a remarkable improvement in his general well-being and gained 15 pounds in 2 months. Three months later the dosage was cut to 25 mg. a day and the blood picture has remained satisfactory at 13.0 Gm. of hemoglobin and 4.5 million red cells. The stools are soft and bowel movements occur two or three times a day. The patient has returned to work and appears well. At no time has he had any evidence of abnormal neurological signs or symptoms.

Comment: This patient presented a sprue-like syndrome which preceded and followed resection of the small and large intestines. Because of sensitivity to liver extract and transfusion reactions he was completely invalided and no form of therapy was available. The clinical and hematological responses to folic acid resolved this problem and he has been able to perform his work and maintain good health. A review of the literature shows no similar case treated with folic acid. None of the sprue patients reported by previous investigators attained normal Hb. and red cell levels.

## DISCUSSION

Cases 2, 3, and 4 may be included in one group because they all presented a single significant feature; namely, a stationary level of the hemoglobin and erythrocytes at a subnormal level, and the progression of signs of subacute combined sclerosis under the continuous administration of folic acid in doses of 25 to 50 mg., daily, by mouth, or 20 mg., daily, intramuscularly. The reticulocyte response in patient 2 was excellent, that in patient 3 quite satisfactory, and that in case 4 submaximal. The initial improvement in appetite and well-being was not maintained when the patients were treated with folic acid alone for periods up to 100 days. It is significant that patient 3 showed a rise in hemoglobin and red cells to normal levels with iron but developed further evidence of neurologic involvement. That folic acid is probably not the antineuritic factor and that some other substance is necessary for complete clinical and neurological remission is indicated by the hematological and constitutional improvement in cases 2 and 3 when placed on a parenteral liver extract in addition to the folic acid. Patients 5 and 6 were placed on folic acid (15 and 50 mg.) daily, orally, and showed reticulocyte responses which were far below those which one would expect with liver extract. Clinical improvement took place, however, and increases in hemoglobin and red cells occurred. When the reticulocytes fell to the pretreatment level the patients were started on intensive liver therapy. Neither patient showed a further reticulocyte rise. Of particular interest in patient 5 was the marked reduction in megaloblasts in the bone marrow 12 days after folic acid therapy was begun. Cases 7 to 11 inclusive represent a group of patients placed on a regimen of varying amounts of folic acid (5 to 10 mg.) plus  $\frac{1}{2}$  unit of liver extract, intramuscularly, daily. Patients 7 and 8 showed no neurological signs or symptoms at the beginning of therapy and after 54 to 119 days there was no evidence of nervous system involvement. The reticulocyte response in patient 7 was more than double that anticipated with liver extract alone, and the red cell count became normal in 41 days. Patient 8 represented a severe test of the combination of drugs because of the advanced arteriosclerosis, pyuria, hemorrhoids, and diverticula of the colon. The reticulocyte peak was below that of a desirable response but there was a sustained rise for 6 days. The blood picture suggested a plateau response of the hemoglobin and red cells. The patient has recently been placed on large doses of liver extract (30 units twice weekly) intramuscularly. Patients 9 and 10 were admitted with mild neurological signs and symptoms which were completely relieved on the combination of 5 or 10 mg. of folic acid plus  $\frac{1}{2}$  unit of liver extract, intramuscularly, daily. The reticulocytes rose to well above anticipated levels and blood pictures showed complete remissions. Patients 7, 9, and 10 are now being maintained with biweekly injections of 15 mg. of folic acid. It is too soon to determine what constitutional or hematological changes have taken place. Patient 11 presented a sustained, rather marked reticulocytosis on the combination of 10 mg. of folic acid and  $\frac{1}{2}$  unit of liver extract, intramuscularly, daily. There was a dramatic improvement in the neurological symptoms and signs.

Patient 12 fell into the class of a sprue-like syndrome. The rapid clinical and hematologic improvement far surpassed that which is ordinarily noted in perni-

cious anemia, and represented the first patient reported in this group to attain and maintain normal blood levels.

Spies<sup>24</sup> has suggested that an enzyme present in the normal gastrointestinal tract and lacking in patients having pernicious anemia is necessary to liberate folic acid from the conjugated form in which it is presumably ingested in the food. Another explanation is based on the observation that rats fed a synthetic diet plus various sulfonamide drugs developed anemia and leukopenia and a simultaneous inhibition in the growth of the coliform bacilli.<sup>28</sup> The cecal contents of such rats contained less folic acid than those of normal rats.<sup>29</sup> It was suggested that these bacteria are concerned in the liberation of free folic acid from its conjugated form. The enzymatic activity of bone marrow in normal persons and in subjects with pernicious anemia in relation to folic acid is at present being investigated by Heinle and his co-workers.<sup>30</sup> It is beyond the scope of the present paper, which deals entirely with clinical reactions, to discuss these highly interesting but as yet controversial matters.

#### SUMMARY

1. Folic acid in daily doses of 15 to 50 mg., orally, or 20 mg. intramuscularly, usually produced a submaximal reticulocytosis in patients with pernicious anemia.
2. In 3 patients the hemoglobin and red cells rose to a level of about 12.0 Gm. and 4.3 million respectively without further rise after 3 months of therapy.
3. Folic acid in the above doses failed to prevent the development or progression of neurological symptoms indicative of subacute combined sclerosis.
4. In 5 patients folic acid in doses of 5 or 10 mg. orally daily combined with  $\frac{1}{2}$  unit of liver extract injected intramuscularly daily produced a reticulocytosis greater than that anticipated from adequate liver extract therapy alone.
5. With combined liver extract and folic acid therapy there was evidence of improvement in the symptoms and signs of subacute combined sclerosis in 3 patients.
6. Folic acid, combined with  $\frac{1}{2}$  unit of liver extract, was found to produce a complete hematological remission.
7. Folic acid, alone or in combination with small doses of liver extract, produced an improvement in appetite and general well-being in patients with pernicious anemia.
8. The possible enhancing effect of liver extract when combined with folic acid cannot be due to the folic acid content of the former since 1 unit of liver extract contains only 0.38 micrograms of folic acid.<sup>31</sup>
9. Folic acid administered to a patient with macrocytic anemia due to faulty postoperative intestinal digestion and absorption, produced a complete remission in the blood picture and a marked improvement in signs and symptoms.

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## ON HOOKWORM ANEMIA

### (APLASTIC ANEMIA IN HOOKWORM DISEASE)

By EUGENE STRANSKY, M.D., AND FLORENCIO N. QUINTOS, M.D.

**H**OOKWORM anemia has been known since the construction of the Gotthard tunnel in Switzerland in the years 1876-80. Perroncito<sup>1</sup> observed that many Italian workers developed a severe and at times fatal anemia, with hookworm infestation. In the following decades hookworm anemia was called miner's anemia because of its occurrence in deep humid mines. Boycott<sup>2</sup> described cases of hookworm anemia in a coal mine in Cornwall, England, in 1901. Later hookworm anemia was observed in other European countries, in America, and in tropical countries, where suitable conditions for hookworm infestation exist.

The etiology of hookworm anemia is well discussed in the papers of Rhoads, Castle, Payne, and Lawson,<sup>3</sup> Payne and Payne,<sup>4</sup> and Andrews.<sup>5</sup> The anemia is due largely to iron deficiency. Napier, Das Gupta, and Mayinuda<sup>6</sup> in British India indicated that, in the absence of dietary deficiency, even a heavy infestation did not produce anemia. Peña-Chavarria and Rotter,<sup>7</sup> in Costa Rica, observed severe anemia in the districts where meat was too expensive for the poor people, while in the districts where meat was plentiful even for the poor, severe anemia did not occur. Brown and Otto<sup>8</sup> found that in the majority of cases in childhood the infested children were "perhaps on the threshold of anemia" and might simply show reticulocytosis.

Hare<sup>9</sup> in India states: "Poverty and ankylostoma are very faithful bedfellows, for poverty implies primitive living conditions which are just ideal for the spread of the parasites. It is possible that the chronic loss of blood caused by ankylostomiasis may just turn the scale against a marrow, which is fighting hard to make bricks with insufficient straw." Johnston and Adams<sup>10</sup> reported 6 cases of severe anemia due to hookworm infestation in pregnancy. It is probable that in these cases the increased demand for iron in pregnancy, together with the constant loss of iron due to the blood-sucking worm, disturbed the equilibrium maintained between blood formation and destruction before pregnancy despite the hookworm infestation. McKenzie<sup>11</sup> emphasized the frequency of vitamin B<sub>1</sub> deficiency in hookworm disease. Hoff and Shaby<sup>12</sup> reported on polyneuritis in hookworm disease. The well-known fact that children, suffering for a long time from hookworm disease, show retardation of physical and mental development, is in favor of the supposition that hookworm infestation may lead to deficiency of resorption of different food constituents.

Ankylostomiasis is widespread in the Philippines. Therefore knowledge of the end results of hookworm infestation and their prevention is of great interest, especially as regards the public health.

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Our cases were observed in the medical and pediatric wards of the Philippine General Hospital in Manila. Most of the cases came from the provinces surrounding Manila.

We observed three types or stages of blood changes in hookworm disease. In the first type the bone marrow is able to maintain the equilibrium between blood loss and production, and there is no manifest anemia. We call this the stage of "compensated anemia." In the second type the production is unable to counterbalance the blood loss, and a hypochromic anemia results. In the third type the bone marrow is exhausted, and aplastic anemia develops.

*The First Type, or "Compensated Anemia."*—We observed a few cases hospitalized for reasons other than anemia, which were discovered to be infested with hookworms on routine examination of the stools. The findings in the peripheral blood were eosinophilia and reticulocytosis without anemia. The bone marrow findings were characterized by increased erythropoietic activity and likewise eosinophilia. Despite a continuous loss of blood, an increased production in the bone marrow was sufficient to maintain the equilibrium between blood formation and blood loss. Of course it would be of great interest to know the exact data of infestation and its duration. It is well known that this early stage of hookworm disease may not develop into real anemia if food and living conditions are adequate. Lowe and Lancaster<sup>13</sup> in Australia recorded 386 cases of hookworm infestation without finding one in which anemia was present.

We studied 33 cases of severe anemia in hookworm disease, 24 of which were males and 9 females. Although the number is very small, the difference in the sex distribution is striking. There were 18 adults, of whom 13 were males and 5 females. There were 15 children, of whom 11 were males and 4 females. The youngest patient was a female of 4 years, the oldest a male of 60 years. Of these 33 cases, 25 belonged in the second stage and 8 in the aplastic anemia stage.

*The Second Type.*—This is characterized by anemia, microcytosis, hypochromia, eosinophilia, and reticulocytosis in the peripheral blood; and eosinophilia and marked erythropoietic activity of the bone marrow. There is more marked erythropoietic activity than in the first type. According to a recent paper of Fenton<sup>14</sup> in West Virginia, hookworm anemia is hypochromic and microcytic, with a red cell count of 1.0 to 3.5 millions per cu. mm. In advanced cases the red cell count may drop to below 1 million. There is usually an eosinophilia of 5 to 15 per cent, although in more advanced cases eosinophilia may no longer be present. In some cases there may be a hypoplastic tendency of the bone marrow, but the condition is still reversible and the patient recovers.

We present the following cases to illustrate the second type.

*Case 1.* A 36 year old male laborer came with symptoms of weakness, chest oppression, headache, and pallor. The disease started 2½ years before admission with bloody diarrhea, tenesmus, and weakness.

Laboratory findings:

(1) Feces examination revealed hookworm eggs + + +, trichuris eggs + +.

(2) Blood:

Hemoglobin..... 6.0 and 6.7 Gm.

Red cell count..... 2.7 and 3.6 millions

White cell count.....	9,000 and 12,400
Reticulocytes.....	9.3%
Platelets.....	300,000
Differential count.....	P—37%, B—3%, E—36% L—20%, Mo—4%*

## (3) Bone marrow:

Total count—262,000; Blast cells—4%, Prom—13.2%, Myel—22.0%, Y—19.2%, B—13.2%, Mature forms—3.2%, L—3.2%, E—21.2% in all stages of development, Mitotic cells—0.4%, ratio of nucleated red cells to white cells = 1.1:1.0

After administration of reduced iron and anthelmintic treatment, the patient improved so much that after a stay of 26 days in the hospital, the anemia was relieved, and he was sent home recovered.

This case demonstrates a moderately severe anemia with marked eosinophilia in the peripheral blood and a high cell count with eosinophilia in the bone marrow. The evidences for a good erythropoietic activity of the bone marrow are the high percentage of reticulocytes in the peripheral blood and the very high ratio of nucleated red cells to white cells in the bone marrow. This is the clinical pattern of most of the cases of the second type.

*Case 2.* A 10 year old boy was admitted with symptoms of rapid, shallow respiration, abdominal pain with nausea and vomiting of blackish, sour, mucoid material. The disease started 10 months before admission with pallor, easy fatigability, and vertigo. The child was poorly nourished and poorly developed. The heart was markedly enlarged. There was a loud systolic murmur over the precordium. The skin was very pale with a subicteric tinge. There were no hemorrhages.

## Laboratory findings:

(1) Feces were positive for ankylostoma ova.

## (2) Blood:

Hemoglobin.....	2.2 Gm.
Red cell count.....	0.68 million
White cell count.....	10,700 and 15,200
Differential count.....	P—78%, B—4.4%, L—15.2%, Mo—2%, E—0
Reticulocytes.....	9.8%
Nucleated red cells.....	3.2 per 100 white cells
Icterus index.....	10 units
Bilirubin (serum).....	0.5 mg. per 100 cc.

## (3) Bone marrow:

Total cell count—34,000 cells per cmm. Blast cells—2.8%, Prom—8.0%, Myel—17.0%, Y—26.8%, B—15.0%, Mature forms—15.5%, L—7.0%, Mo—0.7%, E—6.5%, Mitotic cells—0.7%, ratio of nucleated red cells to white cells = 0.6:1.0

On account of the very serious condition and the severe anemia, a blood transfusion of 200 cc. was administered. After the blood transfusion the child improved considerably. Nine days after the blood transfusion the blood showed:

Hemoglobin.....	67 Gm.
Red cell count.....	1.8 million
White cell count.....	5,700

## \*Abbreviations:

P—polymorphonuclears	L—lymphocytes
B—band forms	Mo—monocytes
Y—young forms	Prom—promyelocytes
E—eosinophils	Myel—myelocytes

Note: Bone marrow materials were obtained by sternal puncture.

Platelet count.....	93,000
Reticulocytes.....	2.9%
Icterus index.....	8 units

After the blood transfusion 4 Gm. of reduced iron was administered daily. The heart murmur soon disappeared. During the third week of his stay in the hospital the boy was up and about. After five weeks he was sent home entirely recovered.

This case showed a tendency to hypoplastic reaction of the bone marrow as shown by the rather low white cell count, the thrombocytopenia, the low cell count in the bone marrow, and the erythropoietic activity which is not so high as compared with the previous case. However, the condition was still reversible and the patient recovered entirely.

The youngest case (a 4 year old girl) and a 33 year old male had a similar hematological picture, but both recovered.

*Case 3.* This was a 12 year old boy in whom there was a simultaneous infection with schistosomiasis chronic amebiasis, ascariasis, and trichuriasis, besides the ankylostomiasis.

*Blood findings:*

Hemoglobin.....	11 Gm. and 12 Gm.
Red cell count.....	3.0 and 3.6 millions
White cell count.....	26,000 and 36,000

In the differential count there were 63.2 and 76.0% eosinophils

*Bone marrow findings:*

Normal count, normal erythropoietic activity with a ratio of nucleated red cells to white cells of 0.3:1.0.

As far as we could ascertain, the anemia was not influenced by the other parasitic infections. In schistosomiasis without hookworm infestation, there is usually no severe anemia, even in the stage of parasitic cirrhosis. Stransky, Jongco, and Pascual<sup>15</sup> examined the blood of 443 apparently normal Filipino children, 70 per cent of whom were infested with ascaris and trichuris. There was no case of anemia among them.

*The Third, or Irreversible Type (Aplastic Anemia).*—This is characterized clinically by severe anemia, dizziness, easy fatigability, and hemorrhagic diathesis. The erythropoietic activity of the bone marrow is almost nil. There is so-called panmyelophthisis with low cell count; relative lymphocytosis; and lack of erythropoietic, granulocytopoietic, and thrombocytopoietic activity of the bone marrow. The clinical symptoms of panmyelophthisis may develop prior to the definite hematological changes. In this stage anthelmintic and antianemic treatments are of no avail. Blood transfusions and iron are ineffective in improving the irreversible process.

While there is an extensive literature in hookworm anemia, aplastic anemia as a possible outcome of hookworm infestation has only rarely been mentioned. Heilig and Wisweswar<sup>16-18</sup> observed several cases of severe anemia in India but without recording one of aplastic anemia. Cruz<sup>19</sup> in Brazil examined the bone marrow of 24 individuals suffering from hookworm anemia. Red bone marrow was observed in 23 cases, and in only 1 case was yellow bone marrow encountered. Diwany<sup>20</sup> in

Egypt found red bone marrow in his 9 cases of hookworm anemia in children. Schretzenmayer<sup>21</sup> in China reported on aplastic anemia in hookworm disease without discussing its development. The observation of fatal anemia in dogs infested with *ankylostoma caninum* (Foster and Landsberg<sup>22</sup>) cannot be compared with the anemia of man as the consequence of massive infestation in dogs is an acute anemia, while human hookworm anemia is chronic.

We have observed 8 cases of aplastic anemia in hookworm disease. There were 7 males and 1 female. During the same time of observation we observed 5 so-called primary idiopathic aplastic anemias. There were 4 females and 1 male. This probably demonstrates that there is no particular sex susceptibility for aplastic anemia, and the preponderance of males here only follows the predominant number of males in the whole series as mentioned above.

We shall now discuss the cases of aplastic anemia.

*Case 4.* The first case of aplastic anemia is a 20 year old male who was admitted with a history of 2 months' weakness and pallor. With these symptoms he noticed simultaneously slight bleeding of the gums. One month later he was hardly able to walk and had palpitations and occasional fever. Two weeks before admission he felt numbness of both legs.

Conspicuous findings on admission were marked pallor, bleeding from the gums, and enlarged heart with functional murmurs.

Laboratory findings:

(1) Blood:

Hemoglobin.....	3 Gm.
Red cell count.....	0.9 million; rose to 1.1 millions after transfusion, then down to 0.7 million
White cell count.....	6,000 and 2,600
Differential count.....	L—56% to 89%, E—7 and 1%
Reticulocytes.....	2.0% and 0.8%
Platelet count... ..	64,000 and 26,000

- (2) Bone marrow was extremely poor in cells. The total counts were: 29,000, 4,200, and 5,300. L—60 to 85%. Ratio of nucleated red cells to white cells was 0.1 : 1.0  
Very rare megakaryocytes seen

Improvement was only temporary after transfusion. The patient left the hospital against advice in very serious condition.

This case was characteristic of chronic aplastic anemia. There were hypochromia, anemia, leukopenia with granulocytopenia, and relative lymphocytosis, thrombocytopenia, and reticulocytopenia in the peripheral blood. There were low cell count, relative lymphocytosis, poor erythropoietic activity, and megakaryocytopenia in the bone marrow. Blood transfusions, large doses of reduced iron, anthelmintic, and dietary treatments did not improve the condition, and the patient was taken home against advice after 5 months in the hospital.

*Case 5.* This second case is similar to case 4. A 15 year old male was admitted with bleeding of the gums and petechial hemorrhages in the skin.

(1) Blood findings were:

Hemoglobin . . . . .	4 Gm.
Red cell count . . . . .	1.2 million

White cell count.....	4,100
Platelet count.....	21,600
Reticulocytes.....	1,2%
Differential count.....	P—3.6%, B—3.2%, L—84.4%, Mo—4.0%, Plasma cells—0.4%, E—0.4%, no nucleated red cells, no anisocytosis

## (2) Bone marrow:

Total cell count—4,450, L—86.4%, ratio of nucleated red cells to white cells = 0.05 to 1.00

Megakaryocytes were not found

The patient stayed in the hospital for 3 months. During this period he received 8 blood transfusions. The red cell counts ranged from 0.68 to 1.39 millions, the white cell counts from 1,800 to 4,800, the lymphocytes from 67% to 89%. Eosinophils were absent. Two days before the patient left, in very serious condition, there developed ulcerations in the tonsils. The hemorrhagic symptoms persisted. The beneficial effects of blood transfusions became less and less and the patient continued to become worse until his discharge against advice in dying condition.

The first two cases discussed were typical of the aplastic anemia following hookworm disease. The diagnosis can be made easily from the clinical picture with the characteristic peripheral blood and bone marrow findings.

The next two cases illustrate a clinical picture in which, while the outcome was the same as aplastic anemia, the hematological picture at first was not definitely that of panmyelophthisis.

*Case 6* is a 10 year old male, admitted with epistaxis, hematemesis, and epigastric pain, which developed 4 hours before admission. For a year the boy had suffered from repeated attacks of epistaxis, hemorrhages from the gums, subcutaneous ecchymosis, and pallor. On admission the child had rapid, weak pulse, cold extremities, and ecchymoses in the skin and subcutaneous tissue of both legs.

## Laboratory findings:

(1) Feces examination: ascaris + + +, ankylostoma eggs + +, trichuris eggs + +

## (2) Blood:

Hemoglobin.....	6.7 Gm.
Red cell count.....	1.5 million
White cell count.....	6,250
Differential count.....	P—43.5%, B—5.5%, L—47.5%, Mo—2.5%, plasma cell—1.0%, no nucleated red cells
Platelet count.....	78,500
Reticulocytes.....	1.5%

## (3) Bone marrow:

Total cell count—78,500

Differential count: Basophils—0.4%, Blast cells—2.4%, Prom—11.2%, Myel—16.4%, L—24%, Mo—1.2%, E—10.4%, mitotic cells—0.8%, ratio of nucleated red cells to white cells = 0.3:1.0

Normal megakaryocytes in number and structure.

The first impression of this particular case was that of a blood dyscrasia, like purpura, primary or secondary. Although the thrombocyte count was low, coagulation and bleeding time were normal and the tourniquet test negative. The severe anemia and the normal megakaryocytes in the bone marrow pointed against primary thrombocytopenic purpura. In spite of repeated blood transfusions there was a marked improvement of the anemia.

The blood findings chronologically were:

Date	Hemoglobin (Grams)	Red cell count (Millions)	White cell count	Ratio nucleated red cell:white cell
8-24-40	6.3	1.5	6,200	0.01 : 1.3
8-27-40	5.6	1.5	7,400	
8-29-40	5.6	1.8	4,800	
8-31-40	5.6	1.35	6,000	
9-5-40	5.6	1.6	6,200	
9-19-40	5.6	1.8	5,500	
9-24-40	5.6	1.8	6,000	0.005 : 1.3
9-27-40	4.9	1.57	5,700	
10-2-40	5.2	1.6	3,000	
10-4-40	5.2	1.56	6,300	

The differential count and the reticulocyte percentage did not change markedly. After the first blood transfusion the eosinophils rose to 13% in the peripheral blood and the bone marrow examination showed a count of 217,000 cells with 22% eosinophils, and a 1:1 ratio of nucleated red cells to white cells.

The child stayed in the hospital for 7 weeks. Reduced iron and ammonium citrate were administered in large amounts; oil of chenopodium was given as anthelmintic treatment. The weight increased from 19.8 Kg. to 22.9 Kg. The hemorrhages disappeared. In spite of the increased erythropoietic activity of the bone marrow, and the gain in weight during the short period of remission, the red cell count and hemoglobin level did not improve. Two weeks after discharge the child was brought back with severe hemorrhages from the nose and gums, and petechial bleedings in the skin. He was pulseless, semi-conscious, and apparently exsanguinated. Blood transfusion was recommended, but the parents refused and he was taken home, where he died a few hours later.

This case is an example of severe anemia with symptoms of early hemorrhagic diathesis. From the hematological picture alone, the diagnosis of aplastic anemia could not be definitely made during the stay of the patient in the hospital for 7 weeks. However, the outcome was fatal and, in 2 weeks after discharge, there was probably a rapid breakdown of the bone marrow. It was unfortunate that no examinations were made during the second admission when the patient was in extremis, as the parents refused further examination and treatment.

*Case 7* is a fatal case of ankylostomiasis with autopsy report, in a 15 year old male. He was admitted with gum hemorrhages, epistaxis, petechial hemorrhages of the skin, bleeding from the right ear, and extreme pallor. Bleeding from the gums started 2 months previously, and epistaxis, 20 days before admission. A few days later skin bleedings developed, weakness and pallor set in.

(1) Blood findings:

Hemoglobin	5.2 Gm.
Red cell count	1.5 millions
White cell count	2,900
Platelet count	14,100
Reticulocytes	0.9%
Differential count	P—29%, B—2%, L—66%, Mo—2%, E—2%, no nucleated red cells

(2) Bone marrow: total cell count—105,000, with E—3.2%.

The other cells were almost normal. Ratio of nucleated red cells to white cells = 0.19:1.00

The patient stayed in the ward for 2 weeks. Unfortunately, a suitable donor could not be obtained, as the cross-matching always revealed hemolysis. The patient became weakened, and paler from day to day. Subsequent examinations revealed a further drop in the red and white cell counts. The hemorrhagic tendency became worse, and retinal bleedings set in. The patient rather suddenly died, probably from heart failure.

The autopsy findings were: anemia, severe, generalized; tigroid heart with dilatation; petechial hemorrhages in the epicardium; hemorrhage and edema of the lungs, endocardium, and cerebellum; yellow bone marrow with reddish (hemorrhagic) areas; purpura of the skin; ankylostomiasis, severe.

In this case the anemia was not extreme at first, with a count above 1 million; the bone marrow count was 105,000, which is about normal, and the differential count was not much altered; but the erythropoietic activity of the bone marrow was low, the ratio of nucleated red cells to white cells was 0.19:1.0 compared to 0.3:1.0; 0.6:1.0; and 1.1:1.0 in those that recovered in the second type. The hemorrhagic symptoms, leukopenia, with relative lymphocytosis, thrombocytopenia, low reticulocyte count, and absence of nucleated red cells in spite of the anemia, shown by the peripheral blood, were in favor of aplastic anemia even in the beginning of the observation, despite the somewhat normal bone marrow findings. Repeated bone marrow punctures in the follow-up would probably have shown more definite aplastic reaction.

The last two cases were similar in that the anemia was not very severe at first, the bone marrow findings were not definitely those of aplastic anemia, and the leukocyte and differential counts were not much altered. Therefore a definite diagnosis of aplastic anemia could not be made at first, typical aplastic anemia later developing. In both cases, hemorrhagic tendency was observed early in the course of the disease. This symptom, when observed in hookworm infestation, may be a danger signal as it was observed in all those cases that died, even before the other findings of aplasia were evident; and it was not observed among the cases of hookworm anemia that recovered. Quintos in an unpublished paper (1943) called attention to this early danger signal in hookworm anemia, and although the number of cases observed is small, it is an interesting point for further study.

The next consideration is: Are these cases of aplastic anemia simply coincidental with hookworm anemia? It is indeed difficult to give a definite answer. During the same period of time we observed 5 cases of so-called idiopathic aplastic anemia, compared to the 8 cases associated with hookworm anemia. The incidence of an aplastic bone marrow response in hookworm anemia is therefore high compared to that without hookworm disease; a definite causal relationship may therefore be present.

The pathogenesis is still a more difficult problem. Our experience is that hookworm anemia is rare in young children. Our youngest case was 4 years old, the next was 7 years old, and all the others were at least 10 years old. On the other hand, it is probable that infestation takes place early, and after anemia has developed, it takes some time more for aplastic anemia to manifest itself. We did not see a case of aplastic anemia below the age of 10 years. We believe therefore that it takes several years before aplastic anemia develops. While the pathogenesis is obscure, we are of the opinion that there is an exhaustion of the bone marrow following continuous blood loss in the presence of various factors, including dietary deficiency. It is known that aplastic anemia does not develop in chronic hemolytic anemia where

there is an abnormal load on the bone marrow to produce red blood cells to counterbalance hemolysis during the whole life of the individual or until therapy is instituted. Therefore it is presumed that some other factor besides the blood loss must play a part in the development of aplastic anemia in hookworm disease.

## SUMMARY

We have discussed three types or stages of blood changes in hookworm disease. Attention is directed to a severe irreversible aplastic anemia which occurs not infrequently in hookworm disease. The pathogenesis is obscure, but we believe that it is due to bone marrow exhaustion following continuous blood loss in the presence of various factors, including dietary deficiency.

The symptomatology, hematologic and bone marrow findings are described.

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# OBSERVATIONS ON THE EFFECT OF IRRADIATION IN CHRONIC ACQUIRED HEMOLYTIC ANEMIA EXHIBITING HEMOLYTIC ACTIVITY FOR TRANSFUSED ERYTHROCYTES

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EVIDENCE is accumulating that transfused cells are destroyed at an accelerated rate in one type of acquired hemolytic anemia.<sup>1-4</sup> Since normal cells as well as the patient's are involved it is evident that a hemolysin is present which is active against all erythrocytes. In the few instances in which a hemolysin has been demonstrated *in vitro* it has exhibited the characteristics of an immune body requiring complement for activity.<sup>5-7</sup> If an antigen, antibody reaction is the basis of hemolysis in the hemolytic anemias showing accelerated destruction of transfused cells it is not surprising that the agent has seldom been demonstrated in serum or plasma, since it is active at body temperature and would therefore be expected to become attached to the red cells as it is released.

Recent investigations on the formation of antibodies have re-emphasized the importance of lymphatic tissue and lymphocytes in antibody production.<sup>8,9</sup> Previous observations by Hektoen<sup>10</sup> and by Murphy and Sturm<sup>11</sup> demonstrated that sufficient irradiation of lymphatic tissue will inhibit antibody formation in animals. It seemed worth while, therefore, to observe the effect of irradiation on the hemolytic anemia in a patient in whom it had been demonstrated that normal red cells as well as her own were destroyed *in vivo* at an accelerated rate. Because irradiation and transfusion were followed by a remission in the severity of the disease a second patient with chronic hemolytic anemia exhibiting similar features was also exposed to irradiation. The results of these observations are reported.

## METHODS

The methods of study have been described previously.<sup>4</sup> In addition, the technic of Ashby<sup>12</sup> was used in following the rate of disappearance of the transfused Group O cells. The patient's blood was Group A Rh positive. Oxalated venous blood was drawn to the 0.5 mark in an erythrocyte pipet and diluted to the 101 mark with a 50 per cent dilution in saline of a high titered (1-10,000) Group B serum. After mixing by shaking 1 minute the sample was allowed to stand 1 hour at room temperature. It was then rotated on an Aloe pipet shaker for 15 minutes before filling the counting chamber. The unagglutinated cells were counted by the usual technic and the number per c.mm. computed. Prior to transfusion the counts of unagglutinated cells averaged 40,000 per c.mm. Following transfusion with Group O cells two counts were made with separate pipets and the average used. With this method variations in two counts were never greater than 10 per cent.

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## CASE REPORT

Mrs. F. A. J., age 58, entered the clinic service of Stanford University Hospital on August 23, 1945, for study of a recurrence of a hemolytic anemia 18 months after splenectomy had apparently resulted in a cure. She was born in California of Portuguese parents. A kyphoscoliosis which had caused her little trouble had been present since birth. Measles and pertussis were the only childhood diseases recalled. She had been married 34 years and had had four pregnancies. Three children are living and well; one died in infancy of an unknown cause. Her father died of diabetes and her mother of tuberculosis. One sister died of uremia and heart failure and another of tuberculosis. Two siblings are living and well. There is no family history suggestive of hemolytic anemia.

She was well until 1939, when she began to have pain in the hips and lower back radiating down the posterior aspect of the right leg. The pain was intermittent but increased so she consulted a physician in November of 1941. The hemoglobin was 77 per cent and the spleen was palpable and tender. She received iron by mouth and intravenously during the next several months, along with injection of arthritis vaccines. In May 1942 the hemoglobin was 8 Gm. per 100 cc. and erythrocytes 2.38 million per c. mm. Moderate variation in size and shape of the red cells was noted. A reticulocyte count was not done, but polychromatophilia was said to be rare. Blood Wassermann, Kolmer and Kline were positive. She received several injections of arsenicals but no consistent antiluetic therapy was instituted. A series of injections of gold sodium thiosulfate was begun on June 8, 1942, for her arthritic symptoms. Because of continuation of her obscure anemia associated with splenomegaly she was referred to the private service of Stanford Hospital on July 20, 1942, for further study.

She was noted to be sallow but not icteric. The sclerae were clear. Pupillary reactions were normal. There was no generalized adenopathy. The heart was enlarged and there was a systolic murmur at the apex and in the pulmonary area. The edge of the spleen was felt 5 cm. below the costal margin and the liver edge was palpable and smooth.

The hemoglobin was 6.17 Gm. per 100 cc. and erythrocytes 1.75 million per c. mm. The leukocytes numbered 3,800 per c. mm. The differential count of leukocytes was normal except for a high banded count and 1 per cent myelocytes. There were 400 nucleated red cells per c. mm. and the reticulocyte count was 20 per cent. Blood platelets were 118,200 per c. mm. The icterus index was 11. Mean corpuscular values were within normal limits. Hypotonic fragility of the erythrocytes was close to normal with hemolysis beginning at .50 per cent salt solution and the control at .46 per cent. There were no microspherocytes in the stained smear, and the average cell thickness was 2.3 micra. The Wassermann reaction was positive although atypical and was reported as follows:

Cholesterinized heart antigen (— —)

Acetone insoluble antigen (+ + +)

Alcoholic extract antigen (+ + +)

The Hinton flocculation was negative.

A diagnosis of hemolytic anemia was made and she was returned to her physician with the suggestion that transfusion therapy be tried for a while.

There was evident improvement for several months following repeated transfusions. However, the symptoms of weakness and fatigue recurred, and while the anemia did not become severe the hemoglobin was 10.64 Gm. per 100 cc. and the reticulocytes 10 per cent at the end of one year. Cell fragility of hypotonic saline was still close to normal. She was readmitted to the hospital on August 16, 1943, and because of the continued signs of hemolytic anemia a splenectomy was done by Dr. Frederick Reichert on August 23, 1943. A bone marrow biopsy taken from the left 11th rib at the time of splenectomy revealed hypertrophy of the marrow stroma and an increased proliferation of erythroblastic cells. The operation was followed by rapid improvement, and the hemoglobin level and erythrocyte count rose to a normal range in 2 weeks' time.

She was well and entirely free of anemia until March 1945, when there was a recurrence of arthritis with swelling of fingers, wrists, and knee joints. During the next 2 months she received gold sodium thiosulfate injections totaling 11 cc. About 3 months after beginning the gold therapy she noted weakness and "pounding of the heart" and shortness of breath on exertion. The above symptoms increased in severity until the time of her third admission on August 23, 1945.

*Physical Examination.*—She was cheerful and able to be up and around her room. The skin and mucous

membranes were pale. The sclerae were not icteric. The pupils reacted well to light and accommodation. The tongue was normal in appearance. There was no generalized lymphadenopathy. The chest was clear to percussion and auscultation. The cardiac rhythm was regular and a systolic murmur was heard over the precordium. Blood pressure was 110 mm. systolic and 70 diastolic. The splenectomy scar was firmly healed. The liver edge could not be felt. Pelvic examination was normal. The extremities, including the joints, were not remarkable and the deep tendon reflexes were equal and active.

*Laboratory Work.*—Examination of the blood gave the following values: hematocrit 24, erythrocytes 2.1 million per c. mm., Hb. 8.6 Gm. per 100 cc., MCV 114 cubic micra, MCH 40 micro micrograms, MCHC 36 per cent, reticulocytes 17.6 per cent, 210 nucleated erythrocytes per c. mm., 60,000 platelets per c. mm., and 7,000 leukocytes. The differential count of leukocytes was polymorphonuclear neutrophils 70 per cent, eosinophils 5 per cent, lymphocytes 18 per cent, and monocytes 7 per cent.

Examination of the stained erythrocytes showed great variation in size with many large polychromatophilic cells and many small densely staining cells and microspherocytes.

The blood Wassermann reaction was negative on this and several subsequent occasions. Total proteins were 7.2 Gm. per 100 cc.

*Course.*—The salient features of her subsequent course until the time of death are shown in figure 1. Because she was comfortable in spite of her anemia it was decided not to give her transfusions until necessary. She was, therefore, allowed to return home after 6 days' hospitalization. Subsequent examinations during the following month showed a drop in hematocrit and an increase in reticulocyte percentage. There was a marked increase in weakness and pallor, and she was readmitted on September 23, 1945, with a hematocrit of 22. The erythrocyte count had dropped to 1.45 million per c. mm. and the hemoglobin to 7.2 Gm. per 100 cc. The reticulocytes had increased to 32 per cent and the icterus index to 30. The urobilinogen output had increased to 2500 mg. per day. The leukocyte count was 12,500 with 21 per cent lymphocytes. There was a slight increase in hypotonic fragility as compared to the previous examination. She exhibited a titer of cold agglutinins of 1-32, but no agglutination could be demonstrated at body temperature. No atypical isohemolysin could be demonstrated in her serum when suspensions of her own or normal cells were incubated in her serum at 37° for 4 to 20 hours.

With an increase in severity of her anemia she complained of more epigastric distress and nausea after taking food or liquids. The gastrointestinal symptoms improved or became more pronounced in relation to the severity of her anemia throughout the rest of her course.

The effect of repeated transfusions of citrated Group O cells is shown in figure 1 and in more detail in figure 2, where the number of unagglutinated Group O cells and the total erythrocyte count before and after transfusion are shown. The number of autologous erythrocytes was computed by subtracting the number of unagglutinated cells from the total number per c. mm. As shown in figure 1 the rise in hematocrit following transfusion was transitory and the drop toward the pretransfusion levels was rapid. As shown in figure 2 the drop in erythrocyte count was due to the rapid disappearance of the transfused cells.

The rapid restoration of the circulating hemoglobin to normal levels by multiple red cell transfusions had no immediate untoward results. Reactions were minimal and for several days following transfusions she felt in a more or less normal state of health. The rapid drop in hematocrit was associated with the recurrence of weakness, shortness of breath, and indigestion. Transfusions became increasingly difficult because of thrombosis of the superficial veins.

As a result of these observations on the accelerated destruction of transfused normal cells it was concluded that a hemolysin must be present which was active *in vivo* but could not be demonstrated *in vitro*. Repeated attempts to demonstrate the presence of a hemolysin in the serum by incubating cell serum suspensions all met with failure. Since it seemed likely that the hemolysin was an immune body type it was decided to see if any measures could be used to modify its production. Accordingly on October 29 she was given 72 cc. of thorotrast (24 to 26 per cent thorium dioxide) intravenously with two purposes in mind. It was thought that the absorption of a large amount of colloidal material by the reticuloendothelial cells might modify the production or elaboration of a hemolytic antibody. Thorotrast was chosen instead of some other colloidal material because of the possibility of demonstrating an accessory spleen which might be removed. There was no reaction to the injection of thorotrast. Films of the upper abdomen showed good visualization of the liver, but no shadow that could be interpreted as an accessory spleen was present.

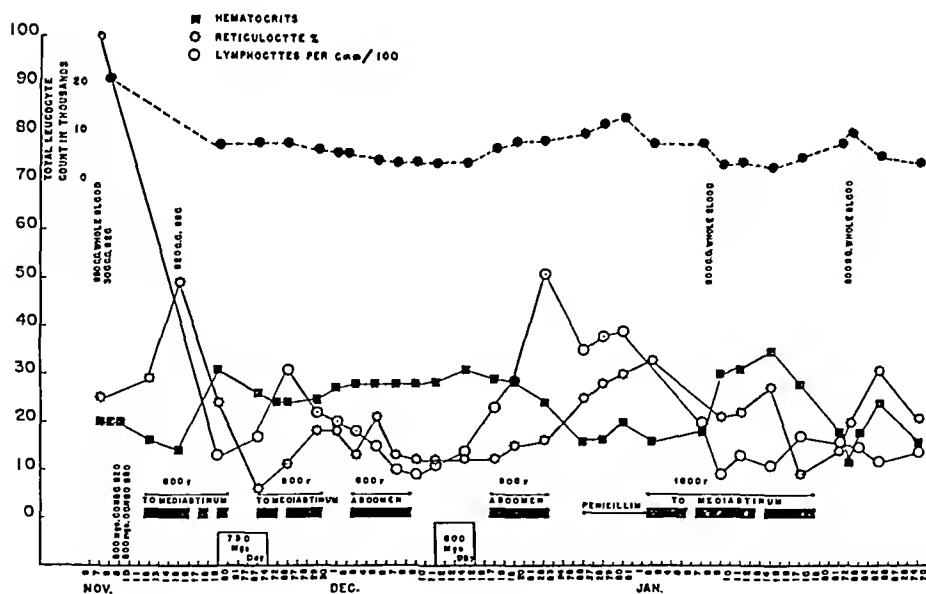
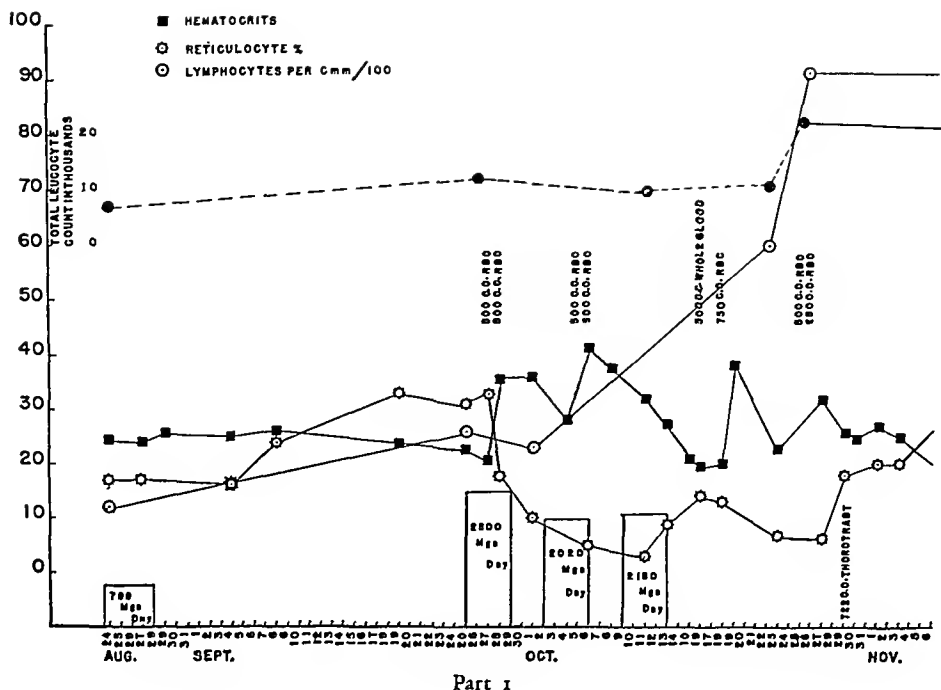


FIG. 1. THE ESSENTIAL HEMATOLOGICAL DATA IN THE COURSE OF THE PATIENT FROM THE TIME SHE WAS FIRST SEEN IN RELAPSE UNTIL THE TIME OF DEATH

The first part covers the period of control observations as the anemia became more severe and the period of multiple red cell transfusions, and finally the injection of thorotrast which produced a transitory interruption in the fall in the hematocrit. The second part shows the effect of irradiation on the leukocyte count and the two periods of remission which were apparently induced by transfusion following irradiation. Determinations of the rate of excretion of fecal urobilinogen at intervals are shown in blocks as mg. per day.

At the time the thorotrast was given the hematocrit was falling following transfusions and judging by previous experience could have been expected to drop further. As can be seen in figure 1, the fall in hematocrit was interrupted and there was a rise from 25 to 27 three days later before the gradual drop was continued. Ten days following injection of thorotrast the hematocrit had reached 20 and she was given 250 cc. of whole blood and 300 cc. of concentrated cells without producing a rise in the hematocrit or hemoglobin. Two injections of 200 cc. of Congo red were given on subsequent days without evidence that the hemolytic process was affected.

On November 12 the hematocrit was found to be 16 and the hemoglobin 4.2 Gm. per 100 cc. Irradi-

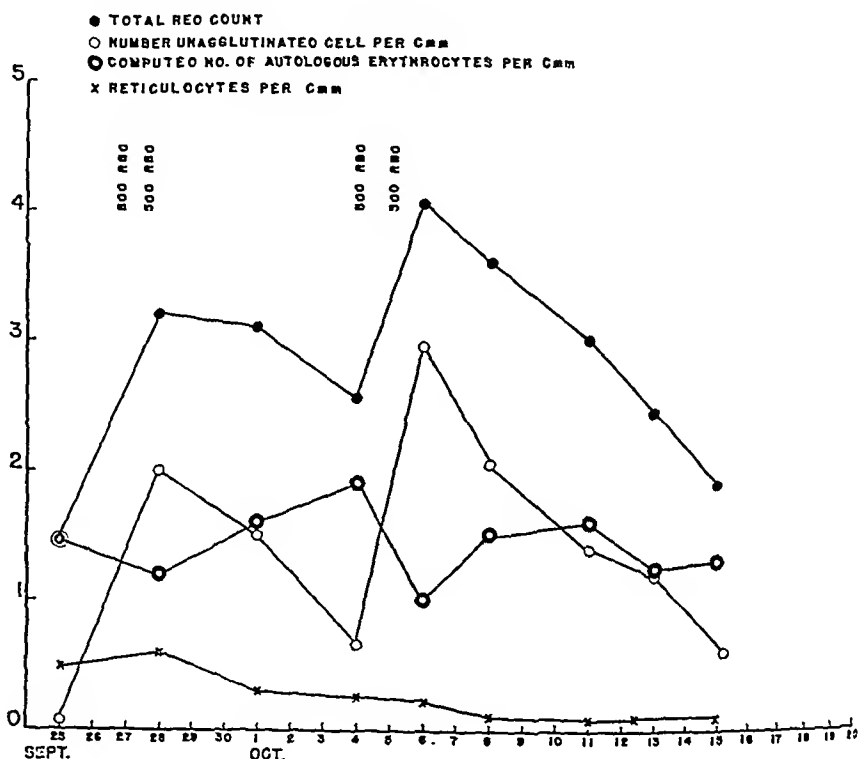


FIG. 2. THE TRANSITORY EFFECT OF MULTIPLE RED CELL TRANSFUSIONS PRECEDING IRRADIATION

This was due in part to the hemolysis of the transfused Group O cells which were counted as agglutinated cells in the Group B serum. The number of patient's cells per c. mm. was computed by subtracting the unagglutinated cell count from the total erythrocyte count. Approximately 50 per cent of the transfused cells disappeared from the circulation in 5 days' time, which is about ten times the normal rate.

ation was begun by giving 100 r in air daily to the anterior mediastinum. The chest was 19 cm. thick. A 12 by 18 cm. portal centered over the mediastinum anteriorly and posteriorly was treated, using radiation of half value layer of 1 mm. of copper. The skin target distance was 70 cm. The same method and dose were used in irradiating the periaortic region of the abdomen in subsequent courses. On the fourth day of irradiation the hematocrit was 14 and the icterus index 40. Her clinical condition was very poor, with extreme weakness, air hunger, and persistent nausea. Oxygen therapy seemed to provide the necessary margin to sustain life. She was given a transfusion of 250 cc. of red cells by cannulating a vein in the ankle. During the next 4 days the improvement was gradual and sustained. Her color improved and she no longer exhibited air hunger. The nausea disappeared and she was able to eat normally. On the

fourth day following transfusion the hematocrit was 31 and the hemoglobin 8.5 Gm. per 100 cc. The icterus index, which had been 40 and 50, dropped to 10 and 15, where it remained during several weeks of remission. The urobilinogen output in the stool was measured as 730 mg. per day during the next 4 days.

The total leukocyte count, which had been 23,000 and 21,200 per c. mm. on two occasions prior to x-ray, dropped to the neighborhood of 8,000. The decrease in circulating lymphocytes and the total leukocyte count are presented in figure 1. Platelet counts varied between 100,000 and 200,000 per c. mm. throughout her course.

When the hematocrit was found to be 31 irradiation was discontinued for several days but was begun when the hematocrit fell to 26. Following the resumption of irradiation of the mediastinum and later of

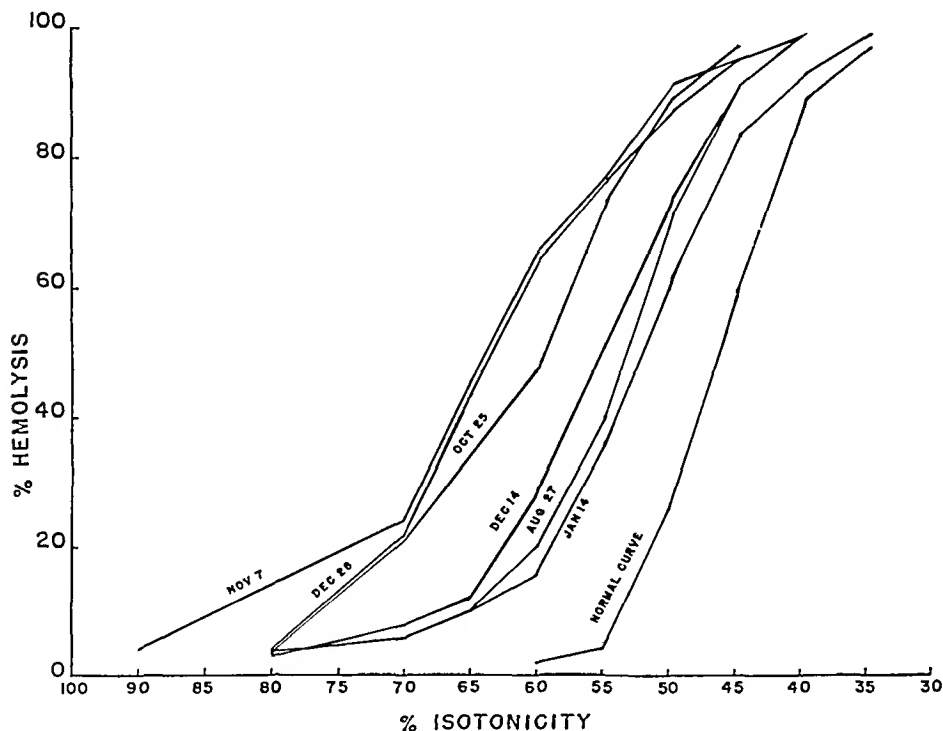


FIG 3. REPRESENTATIVE SAMPLES OF HYPOTONIC FRAGILITY CURVES DURING THE COURSE OF THE RELAPSE IN THE HEMOLYTIC ANEMIA

The increase in hypotonic fragility from August 27 to November 7 during the increase in severity of the disease is evident. Curves obtained on December 14 and January 14 during the remissions show a shift toward normal. The curve of December 26 was done during the period of exacerbation.

the abdomen the hematocrit rose again and the lymphocyte count, which had shown a temporary rise, fell to lower levels. She continued to feel a great deal better and regained sufficient strength during the period of partial remission to be ambulatory. The improvement in the anemia was accompanied by signs of diminished hemolysis. The icterus index remained at a level of 10 to 15 and the urobilinogen output in the stool was less than half the three previous determinations.

The curve of hypotonic fragility, which had shown a gradual increase since the first determination on August 27 along with increasing severity of the hemolytic process, showed a reversal of this trend and shifted back toward the normal stage. The curve of December 14 (figure 3) is a sample of those obtained during the period of partial remission.

Irradiation of the periaortic region of the abdomen was undertaken on two occasions using the same

amount and size of portal as the mediastinum. It was during the second course of irradiation to the abdomen that she exhibited a rise in the number of lymphocytes in the circulation and a precipitous fall in the hematocrit and a return of her symptoms of weakness and nausea. With the drop in the hematocrit she exhibited a low grade fever and developed a mild productive cough which persisted several days. A chest x-ray on December 28 showed the lung fields to be clear. Urinalysis at the same time showed a transient pyuria, but there were no symptoms of urinary tract infection. She was given 30,000 units of penicillin every 3 hours for 7 days. This medication was accompanied by a subsidence of the cough and clearing of the urine sediment, but no improvement in the anemia. Irradiation of the mediastinum was resumed on January 2, 1946. By January 7 no significant improvement in the anemia had occurred, and she seemed unable to continue the struggle longer. A transfusion of 500 cc. of whole blood was followed again by a dramatic improvement. The hematocrit rose from 18 to 30 and then to 34 during the next 5 days, and the hemoglobin rose from 4.9 Gm. to 10.4 Gm. per 100 cc. She again felt greatly improved.

However, on January 16 she complained of pain in the right anterior chest and the following day a friction rub was heard. An x-ray of the chest failed to reveal any area of consolidation. Following this episode, however, the hematocrit fell rapidly and all the former symptoms of severe anemia returned. As can be seen in figure 1, this drop occurred along with continued irradiation. A transfusion of 500 cc. of whole blood was of temporary benefit only, and she died on January 29 with symptoms of marked air hunger.

*Pathological Examination.*—Spleen: The spleen, removed 29 months prior to death, weighed 955 Gm. The capsule was smooth and translucent. Two small infarcts were present. The malpighian bodies were large but not distinct and showed considerable hyaline homogeneous material in the centers. The reticulo-endothelial cells were laden with brownish pigment (hemosiderin) but the phagocytosis of red cells was not prominent.

*Autopsy.*—Gross: There was no free fluid in the serous cavities. The heart weighed 375 Gm. and showed normal values. There were no gross infarcts in the lungs but there were multiple small emboli in the pulmonary arteries, several of which were adherent to the vessel wall. Mediastinal lymph nodes appeared grossly normal. The liver weighed 1950 Gm. and appeared normal. Gallstones were not present. No accessory spleen was found. The adrenals were normal and the kidneys weighed 185 and 200 Gm. The uterus and adnexa were normal, as were the stomach and intestines. The femoral, sternal, and vertebral marrow was a deep red.

*Histological Examination.*—The multiple thrombi in the pulmonary arteries were in various stages of organization and recanalization. Others were more recent but none was found that could be considered of fresh occurrence. The lymph nodes of the anterior mediastinum showed some diffuse fibrosis but no typical radiation fibroblasts were found. The marrow showed hyperplasia of all elements and marked erythroblastic activity. Phagocytosis of red cells was prominent and many macrophages were filled with granules of thorium dioxide. There was a fine diffuse fibrosis of the sternal marrow and some evidence of an arrest of development of marrow elements.

The Kupffer cells of the liver were filled with refractile grayish granules of thorium dioxide. Iron stains showed a moderately heavy deposit of iron pigment in the liver cells and Kupffer cells. The kidneys showed hemosiderin pigmentation of the tubular epithelium.

The mucosa of the esophagus and cardiac end of the stomach was ulcerated in

patchy areas and replaced by young granulation tissue and infiltrated with inflammatory cells. The remainder of the gastrointestinal tract was normal!

*Irradiation of a Second Patient with Chronic Hemolytic Anemia.*—The second patient with chronic acquired hemolytic anemia and spherocytosis to be studied for the effect of irradiation has been reported previously.<sup>4</sup> She exhibited a marked increase in cell fragility which made it possible to demonstrate that transfused cells were made abnormally fragile to hypotonic solution within 24 hours after injection.

Since the partial remissions in the first patient followed transfusions as well as irradiation, it was desirable to determine first what effect if any irradiation alone

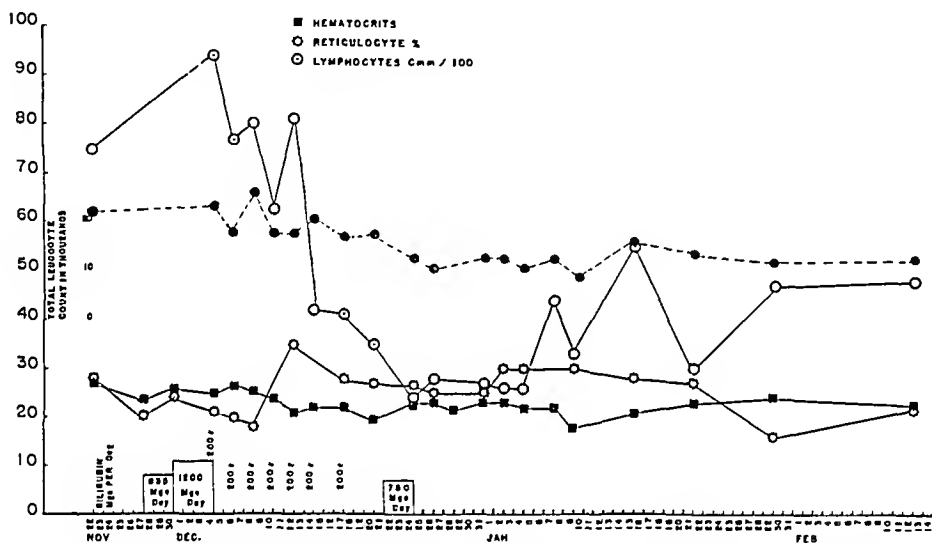


FIG. 4. THE COURSE OF THE HEMOLYTIC ANEMIA IN THE SECOND PATIENT BEFORE, DURING AND FOLLOWING IRRADIATION

There was a drop in leukocytes and lymphocytes but no evidence that the hemolytic process diminished in severity. Determinations of the excretion of bilirubin from the ileostomy are demonstrated in blocks as mg. per day.

would have on the hemolytic process. During the 12 day period of observation the hematocrit varied between 24 and 27, and the output of bilirubin in the ileostomy excreta was measured as 835 and 1200 mg. per day. The effect of 200 r daily in air to the mediastinum given every other day on the total leukocyte and lymphocyte count is shown in figure 4. The reduction in circulating lymphocytes was marked, but there was no measured effect on the hematocrit or bilirubin output to indicate a slowing of the hemolytic process. If there was any significant effect it was in the direction of increasing the degree of anemia temporarily. Five months after irradiation the hematocrit was 26, the leukocytes 12,000 per c. mm., and the lymphocytes 4,000 per c. mm.

## DISCUSSION

In a previous report evidence that injections of colloidal gold had precipitated the onset of the abnormal hemolytic process in one patient was presented. In the case history presented here the relationship of gold therapy to the onset of the hemolytic process is doubtful, since an anemia and palpable spleen were noted before gold therapy was begun. However, the relapse 20 months following splenectomy coincides closely with the resumption of gold therapy. Following splenectomy, the hemoglobin, erythrocyte count, and reticulocyte percentages were normal in all determinations up to the time gold therapy was resumed. The fall in hemoglobin thereafter was evidently gradual, since the onset of pallor and weakness was insidious. The anemia increased in severity until it finally became necessary to resort to transfusion therapy. It is evident that gold therapy must be considered capable of setting off an abnormal hemolytic process.

Histological examination of the spleen and the subsequent autopsy findings failed to throw any new light on the pathogenesis of the disease. The phagocytosis of the red cells by the macrophages of the marrow, although extensive, does not account for a process that damaged the majority of the mature red cells present in the circulation. On the contrary, the phagocytosis was probably stimulated by the presence of the damaged cells. It is likely that the multiple small pulmonary emboli resulted from the thrombosis of superficial veins following transfusions, since no other source was found. The acute ulcerations of the esophagus and stomach account for the epigastric symptoms which were pronounced when the anemia was severe, which suggests that anemia and anoxemia played a role in their production.

Kracke and Hoffman<sup>13</sup> have previously reported the association of positive serology of atypical behavior and chronic acquired hemolytic anemia. In their patient, too, the serological reactions reverted to normal following splenectomy.

According to the method of differential agglutination the transfused Group O cells disappeared from the circulation at roughly ten times the normal rate,<sup>1</sup> since approximately 50 per cent of the cells were eliminated in 4 to 5 days' time. This rate of hemolysis of transfused cells is consistent with the pyrrole pigment excretion, which was somewhat greater than ten times the normal rate. It is obvious that the transfused red cells must have participated in the increased hemolysis in that repeated multiple transfusions which in some instances nearly doubled the total cell volume continued to have a transitory effect. Also, there was evidence from studies of the quantitative fragility curves before and after transfusion that the cells introduced showed a gradually increasing susceptibility to hemolysis in hypotonic solutions during the 3 or 4 days following their introduction. All these observations which show that normal cells were damaged and hemolyzed at an accelerated rate indicate the presence of a hemolysin in the patient's circulation. It is of course only an assumption that the hemolysin was an immune body, but in the absence of other evidence that seems to be the most likely possibility. It is unlikely that the cold agglutinins played a significant role in the hemolytic process since the titer was relatively low and there was no activity at body temperature.

§ The temporary rise in hematocrit that followed the injection of thorium dioxide may be significant since it was the first interruption in the drop in hematocrit following transfusion that had been recorded. There was nothing to suggest that the temporary rise in hematocrit was due to hemo-concentration following the injection. The only direct evidence that the rise was due to a slowing of the rate was a fall in the icterus index from 50 to 30 and then an increase to 50 as the hematocrit began to drop again. There are several possible explanations which might account for a temporary slowing of hemolysis after the injection of a large amount of colloidal material. Filling the reticulo-endothelial cells with colloidal particles might interfere temporarily with phagocytosis of damaged cells and allow more to remain in the circulation. On the other hand the colloidal particles could interfere directly with the hemolysin or form a protective coating around the erythrocyte. Lastly, the absorption of colloidal material might modify temporarily the rate of production or elaboration of hemolytic antibody by the reticulo-endothelial cells. The evidence that the reticulo-endothelial system is a source of antibodies is controversial, but the possibility has not been excluded by recent emphasis on the importance of the lymphatic tissue.

The injection of Congo red was apparently without effect. Congo red has been shown by Richardson<sup>14</sup> to protect erythrocytes against hemolysis by a variety of agents, but the concentration of the dye used in these *in vitro* experiments was much greater than would be achieved in intravenous injection of 200 mg. It is noteworthy that Congo red, unlike thorium dioxide, is not, according to observations in animals, absorbed by the reticulo-endothelial cells alone.<sup>15</sup>

The prolonged partial remission which followed the single transfusion given after 4 days of irradiation showed that either the irradiation or some other completely unknown factor had modified in a radical way the severity if not the nature of the hemolytic process. The most obvious effect of the irradiation was the depression of the number of circulating leukocytes, particularly the lymphocytes, which is in agreement with the studies of Minot and Spurling<sup>16</sup> on the effect of x-ray therapy. As can be seen in figure 1 there seemed to be a rough inverse relationship between the number of circulating lymphocytes and the degree of severity of the anemia until the terminal 2 weeks of her course. The leukocytosis and lymphocytosis that preceded the period of irradiation had no obvious clinical explanation but seemed to be associated with the multiple red cell transfusions and followed a period of increased blood breakdown.

It is possible theoretically that destruction of large numbers of circulating lymphocytes would increase temporarily the rate of red cell destruction by releasing hemolytic antibody. The evidence that that occurred is equivocal. As shown in figure 1, the hematocrit fell from 16 to 14 during the drop in lymphocyte count and the reticulocytes increased from 29 to 50 per cent, but the severity of the anemia was increasing before irradiation was begun. It was not possible to obtain accurate studies of pigment output during this period. The second patient showed a lowered hematocrit during the drop in circulating lymphocytes but here, too, this degree of variation has been observed to occur spontaneously.

The first partial remission ended with the onset of symptoms which suggested

a respiratory infection and the second with an episode which seemed likely to have been a pulmonary embolus. The drop in hematocrit at the end of the first remission was preceded by a rise in lymphocyte count and a leukocytosis, which suggests that an infectious process may increase the severity of hemolysis by activating lymphatic tissue and increasing the production of a hemolysin.

It is obvious that both partial remissions observed in the patient were initiated by transfusions although in each case the hematocrit rose to a higher level than could have been accounted for by the transfusion alone. The failure to produce a remission in the second patient, who seems to have had a closely similar hemolytic process, with irradiation alone supports the importance of the transfusions in initiating the remissions. There is no good explanation as to why transfusions were necessary to initiate a sustained remission. It is conceivable that an antihemolytic substance in plasma<sup>17</sup> became effective at this point or that the remaining hemolytic antibody was absorbed by the normal transfused cells. Future studies should, if possible, include observations on the effect of plasma and washed red cell transfusions following irradiation.

As already indicated, marked changes in erythrocyte morphology and fragility in hypotonic solutions were observed during the course of the hemolytic anemia. Prior to splenectomy, when the hemolytic anemia was relatively mild, hypotonic fragility was normal as measured by the usual technic. The mean cell thickness, computed by the formula  $\frac{MCV}{\pi \left( \frac{MCD}{2} \right)^2}$ , was 2.3 micra and microspherocytes were

absent in the stained smear. However, when first seen in relapse the blood smear showed a large percentage of spherocytes, the M.C.T. had increased to 3 micra, and the fragility in hypotonic solution was increased as shown in figure 3 (August 27). As the hemolytic process accelerated, the fragility curve became more abnormal (October 25 and November 7), while with each remission the curve shifted back toward normal (December 14 and January 14). The correlation of the hypotonic fragility, which reflects the degree of spherocytosis,<sup>18</sup> with the severity of the hemolytic anemia agrees with findings of Dameshek and Schwartz,<sup>19</sup> who demonstrated that the degree of spherocytosis and the severity of the hemolytic process in experimental hemolytic anemia were proportional to the amount of anti-erythrocyte serum injected.

#### SUMMARY

The history and course of a middle-aged woman with a chronic acquired hemolytic anemia have been presented. Splenectomy resulted in a complete remission of the disease for 18 months before a relapse occurred which led eventually to fatal termination.

The original cause of the abnormal hemolytic process is not known, but the onset of the relapse was closely associated with the resumption of gold therapy.

The acceleration of the hemolytic process was associated with an increased tendency toward spherocytosis and susceptibility of the erythrocytes to hemolysis in hypotonic solution.

It is demonstrated that transfused normal erythrocytes shared in the hemolytic process and were eliminated from the circulation at an abnormal rate although no atypical isohemolysin was demonstrated by *in vitro* tests.

An attempt was made to alter the rate of hemolysis in several ways. The injection of thorium dioxide was followed by signs of transitory slowing of red cell destruction. Injections of Congo red were apparently without effect. Irradiation of the mediastinal and periaortic nodes was begun to ascertain if the production of a hemolytic antibody in lymphatic tissue could be altered. Irradiation was followed by a fall in leukocyte and lymphocyte counts in the peripheral blood. Transfusions were then followed by partial remissions in the hemolytic process for varying periods of time in two instances. Irradiation of a second patient with a chronic hemolytic anemia of similar character produced a fall in leukocyte count and lymphocyte count, but in the absence of transfusions no slowing of the hemolytic process occurred.

No definite conclusions can be drawn from these fragmentary observations on the effect of irradiation, but further investigation as to the character of the hemolytic substance and possible methods of modifying its production are indicated when the opportunity is afforded.

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# CASE REPORT

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## CONGENITAL HYPOPLASTIC ANEMIA ASSOCIATED WITH MULTIPLE DEVELOPMENTAL DEFECTS (FANCONI SYNDROME)

### REPORT OF A CASE

By S. ESTREN, M.D., JOHN F. SUESS, M.D., AND WILLIAM DAMESHEK, M.D.

THE pathogenesis of congenital hypoplastic anemia is not clear. In many cases, the disorder occurs sporadically in a child who is otherwise completely well; in the absence of other etiologic agents, these cases are assumed to result from a chance defect in the chromosomal mechanism for development of the bone marrow. That this hypothesis is probably valid is suggested by the occurrence, in other individuals, of congenital aplasia of the bone marrow in siblings,<sup>1</sup> and also by the occurrence of cases in which the marrow abnormality is merely one of a number of congenital aberrations. Fanconi was the first to describe examples of the latter disorder. In 1927 he reported three brothers aged 5, 6, and 7 years respectively, each of whom showed aplastic anemia, microcephaly, testicular hypoplasia, convergent strabismus, exaggerated deep tendon reflexes, and a generalized brown melanin-like pigmentation of the skin.<sup>2</sup> Familial cases of this "Fanconi syndrome" were subsequently described by Émile-Weil,<sup>3</sup> Hjorth,<sup>4</sup> and Dacie and Gilpin.<sup>5</sup> Sporadic cases of the same disorder were reported by Uehlinger,<sup>6</sup> van Leeuwen,<sup>7</sup> and Émile-Weil.<sup>3</sup>

The purpose of the present paper is to describe an example of Fanconi's syndrome in an 11 year old American-born child. As far as we can determine, this is the first such case to be reported in the American literature. Hypoplastic anemia was associated with pigmentation of the skin, deafness, congenital heart disease, and congenital deformities of the thumbs and forearms.

### CASE REPORT

H. T., an 11 year old white girl, was admitted to the Boston Floating Hospital (No. 9474) on September 10, 1946,\* for investigation of anemia.

*Family History.* Both parents were born in France of French ancestry. The father had never had any illnesses except for unknown childhood diseases over 30 years before the patient was born. He had lived in the United States since 1924 and was 38 years old at the time of the pregnancy. The mother was well

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\* Referred by Dr. Earl S. Kelly, Pawtucket, Rhode Island.

From the Blood Laboratory of the J. H. Pratt Diagnostic Hospital and the Boston Dispensary, the Boston Floating Hospital and Tufts College Medical School. Aided by grants from the Charlton Fund and the Upjohn Company.

before the pregnancy except for a transient episode of "jaundice" which occurred at the age of 12 (France), and since which time she was supposed to be anemic. Actual study of the mother showed no evidences of anemia, spherocytosis, or other defect of the hematopoietic system. The mother had lived in the United States since 1924 and was 33 years old at the time of conception. During the third month of the pregnancy, she suddenly developed faintness and dizziness, was told she had a severe "otitis media and mastoiditis," and underwent mastoidectomy. The subsequent course of the pregnancy was uneventful.

There was no history in the family of either mother or father of blood diseases, spleen disease, anemia, congenital abnormalities, pigmentation of the skin, or other similar disorders.

*Birth History.* The patient was an only child. The mother had never been pregnant before, and after discovering the abnormalities in her daughter refused to become pregnant again. The child was born at home of a vertex delivery in which low forceps were used. At the time of birth, it was noted that the right thumb was lacking and that the left thumb was represented by a single bony rudiment hanging by a thread of skin from the left metacarpus.

*Past History.* Development, in regard to weight gain and mental ability, appeared to be normal. At the age of 3 weeks the patient had pneumonia; but she was well from that time until the age of 4 years, when she developed rubella; at 4 years, she had transient "pyelitis." After the first year, her general development was poor and she was frailer and smaller than other children of the same age.

*Present History.* In 1943, when the patient was 7 years of age, it was noted that she was extremely pale. At the same time, her skin was noticeably darker than previously, and she seemed to have some difficulty in hearing with her left ear. Pallor and increasing pigmentation continued to be present from that time on. In May 1945 the child developed fever, coryza, and cough and was considered to have bronchitis. Sulfadiazine was given, 1.0 gram every four hours for three days, with some relief. The episodes of fever, coryza, and cough which were interpreted as bronchitis recurred in July 1945, December 1945, and February 1946; on each occasion they were treated with similar dosage schedules of sulfadiazine, with regression of the complaints. In February 1946, however, fever continued to be present after the usual course of sulfa therapy, and another similar course of sulfadiazine was given and was after the first course with good response.

In March 1946 the pallor became marked and the child was weak, listless, and unable to carry on her usual activities. A laboratory test was performed at this time and showed anemia. Liver and iron therapy were instituted without effect, and the patient was given two transfusions of whole blood, 500 cc. each. She felt and looked better after this treatment, but in July 1946 again became listless, pale, and weak. Fever of 100° to 102° F. was present at this time and spontaneous ecchymoses began to appear in various parts of the body. On several occasions, spontaneous bleeding also occurred from the gums. The stools were inconstantly streaked with blood. Occasional hematuria was also noted.

*Physical Examination.* The child was a dull-appearing, well nourished, poorly developed white girl. The skeletal development was that of a 6 or 8 year old child.

*Skin.* There was a generalized brown pigmentation of the skin over the entire body, but the oral mucosa was not pigmented. Purpuric spots were present over the abdomen, legs, and arms.

*Ears.* Grossly, the ears were normal. Otoscopic examination showed normal drums. There was, however, definite impairment of hearing in the left ear.

*Eyes.* Normal. No evidence of muscle imbalance.

*Mouth.* Dentition corresponded to the age of 10 years. The tongue, oral mucosa, palate, and throat were normal. There was no abnormal pigmentation.

*Neck.* Normal.

*Breasts.* Although the nipples were not developed, the fatty portions of the breasts were definitely increased over normal and corresponded to the development of a 14 year old girl.

*Chest.* A slight pigeon-breast deformity was present.

*Lungs.* Normal.

*Heart.* The heart was enlarged to both the right and the left. There was a systolic thrill over the left border of the heart, best felt at the left sternal border over the second and third left intercostal spaces. Over the same area a very loud machinery-type murmur was present. It was continuous throughout systole and diastole but was accentuated in systole, and not well transmitted elsewhere. The murmur was well heard in the interscapular area posteriorly. A softer systolic blowing murmur was present

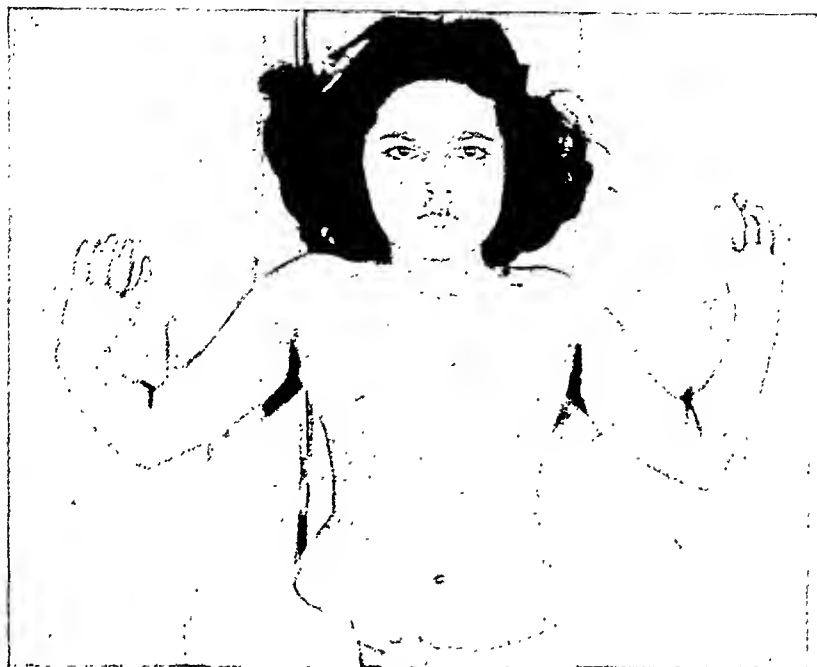


FIG. 1. GENERAL APPEARANCE OF PATIENT

Note the shortening of the right forearm, the absence of the right thumb, and the rudimentary left thumb.

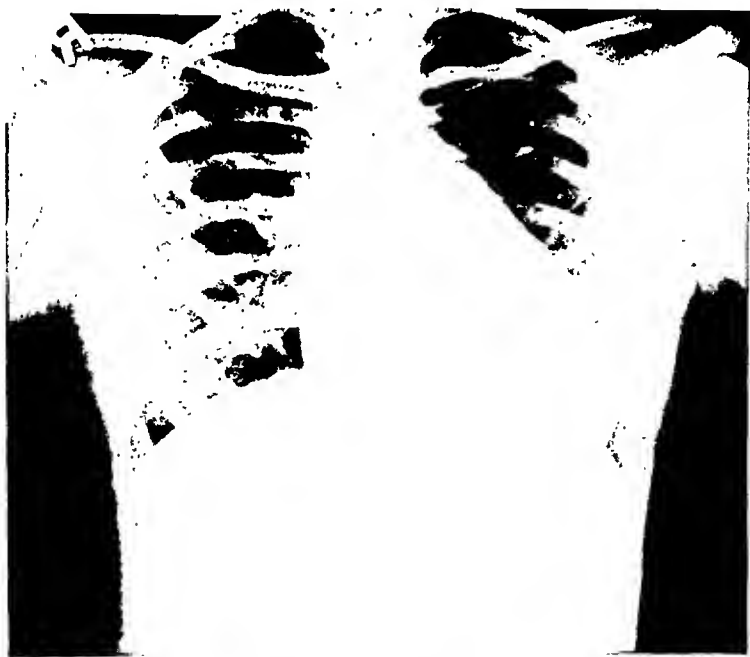


FIG. 2. POSTERO-ANTERIOR X-RAY OF THE HEART

The heart shows enlargement of the left ventricle and the pulmonary conus. On fluoroscopy, the left auricle was also seen to be enlarged.

the apex, transmitted to the left sternal border and the left axilla. The sounds were of good quality and the rhythm was regular.

Abdomen. The liver and spleen were not palpable.

Genitalia. Normal female.

Extremities (fig. 1). The right thumb was completely lacking. The left thumb consisted of a single phalanx which was attached to the left metacarpus by a thread of skin and soft tissue. There was shortening



FIG. 3. X-RAYS OF THE FOREARMS AND HANDS

The right radius and ulna are shorter than those on the left. The distal epiphysis of the right radius is absent. The right hand is deviated radially. The right thumb is absent. The left thumb is underdeveloped. The number of carpal centers of ossification is less than normal.

ing of the right ulna and radius, with deviation of the radial half of the forearm toward the radial side.

*General Laboratory Findings.* The urine showed no abnormalities. The serology of the blood was negative. An electrocardiogram showed no abnormalities. X-ray and fluoroscopy of the chest showed a prominent pulmonary artery, enlargement of the left ventricle, and enlargement of the left auricle (fig. 2). X-rays of the skeletal system showed the following findings (fig. 3):

1. The right thumb was absent.

TABLE I.—*Blood Studies on H. T. before and after Splenectomy*

Date	R.B.C. (millions per cu. mm.)	Hemo- globin (grams per 100 cc.)	W.B.C. (per cu. mm.)	Differential count of white cells	Platelets (per cu. mm.)	Reticulocytes (per cent)
Sept. 11	0.73	2.7	2,700	P 620 (23%) L 1800 (67%) M 270 (10%)	21,000	11.1%
Sept. 13	1.04	2.7	2,450	P 760 (31%) L 1400 (56%) M 290 (12%)	13,500	9%
Sept. 17	1.09	3.4	1,850	P 350 (19%) L 1300 (71%) M 190 (10%)		
Sept. 20	1.94	5.8	2,200	P 310 (14%) L 1600 (74%) M 240 (11%)		
Sept. 24	Splenectomy					
Sept. 25	2.41	7.4	3,450	P 1900 (56%) L 1000 (30%) M 500 (14%)	22,000	12.5%
Sept. 26	2.51	6.6	3,900	P 2000 (51%) L 1400 (36%) M 500 (13%)	22,600	4.3%
Sept. 28	2.52	6.7	2,900	P 1300 (45%) L 1250 (43%) M 350 (12%)	28,000	
Sept. 30	2.40	5.8	2,300	P 1100 (46%) L 1200 (51%) M 50 (2%)	17,000	1.4%
Oct. 4	2.19	6.9	2,150	P 470 (22%) L 1500 (70%) M 130 (6%)	20,000	2.0%
Oct. 8	2.47	6.6	2,300	P 350 (12%) L 1800 (77%) M 150 (7%)	63,000	2.7%
Oct. 26	2.22	7.4	5,000	P 550 (11%) E 100 (2%) L 3800 (76%) M 550 (11%)	15,500	4.4%
Nov. 23	2.49	9.0	5,850	P 940 (16%) L 3500 (60%) M 1170 (20%)	20,000	

2. The right wrist contained only 5 carpal centers of ossification (normally at this age, 8 centers are visible on x-ray).
3. The right radius and ulna were shorter than those on the left.
4. The right radius was shorter than the right ulna. The distal epiphysis of the right radius was absent.
5. The right hand was deviated toward the radial side.
6. The left thumb was underdeveloped.

**Hematologic Findings.** The blood counts are recorded in table 1. On admission, the red blood count was 730,000 per cu. mm.; the hemoglobin 2.7 grams per 100 cc. (18 per cent); the white blood count 2,700 per cu. mm., including 23 per cent polymorphonuclear neutrophils (620 per cu. mm.), 67 per cent lymphocytes (1,800 per cu. mm.), and 10 per cent monocytes (270 per cu. mm.). The platelets numbered 21,000 (normal 300,000) per cu. mm. The reticulocytes numbered 11 per cent of the total number of red cells. The blood smear showed variations in size and shape of the erythrocytes, some hypochromia, and virtually no platelets.

A bone marrow puncture showed hypocellularity. Megakaryocytes were virtually absent from the preparations. Granulocytopoiesis was qualitatively normal, but quantitatively reduced. Erythropoiesis was normoblastic in type and orderly in development, but also quantitatively much reduced. There was a slight relative increase in the numbers of lymphocytes and plasma cells. Differential count of the marrow cells gave the following results:

Blasts . . . . .	0.4%	Erythrogonos . . . . .	0.4%
Promyelocytes . . . . .	1.6%	Normoblasts (basophilic) . . . . .	3.0%
Myelocytes . . . . .	2.0%	Normoblasts (polychromatophilic) . . . . .	10.0%
Band forms . . . . .	8.2%	Normoblasts (orthochromatic) . . . . .	50.2%
Polymorphonuclears . . . . .	6.4%	Ratio of granulocytes to erythroblasts =	
Lymphocytes . . . . .	10.6%		1:2.5 (normal 2.5:1)
Plasma cells . . . . .	3.0%		
Histiocytes . . . . .	4.2%		

**Course in Hospital.** There was no response to liver and iron therapy. Three transfusions of blood totaling 700 cc. were given on September 12, 17, and 18 and had little effect on the symptoms of weakness and fatigue or on the blood values (table 1). Because the patient was going progressively downhill, and because splenectomy has occasionally given beneficial effects in hypoplastic anemia,<sup>1</sup> removal of the spleen was undertaken on September 24. An additional 550 cc. of blood were given at this time. The spleen weighed 39 grams and showed large numbers of follicles. The sinuses were conspicuous. There was diffuse but slight hemosiderosis. No evidences of hematopoiesis were present. Vessels, capsule, and trabeculae were normal.

The patient's convalescence was uneventful. One month postoperatively there were no essential changes in the neutrophil or platelet counts, although a slight lymphocytosis had resulted in a total white count of 5,000 (table 1). The red count level was at a higher level than the patient's pretransfusion levels (2.2 M. as compared to 1.0 M.). Although the role of splenectomy in this regard could not yet be definitely stated, it seemed that the patient was able to maintain a higher level of erythrocyte count than before splenectomy.

#### DISCUSSION

The presentation of this patient as an example of the syndrome described by Fanconi is based upon the co-occurrence of multiple congenital abnormalities including hypoplasia of the hematopoietic system. The patient showed the following defects:

1. Skeletal system. There was underdevelopment of the bones, so that the skeletal age was two to four years less than the chronological age. There was

hypogenesis of the left thumb and agenesis of the right thumb and two carpal bones of the right hand. There was maldevelopment of the bones of the right forearm.

2. Central nervous system. There were no obvious abnormalities of the nervous system, with the exception of the deafness of the left ear. The etiology of this deafness was obscure; although it had been definitely noted only in the three years before admission, the parents recalled that the child always had some difficulty in hearing, so that it is likely that the deafness too was a developmental abnormality.

3. Cardiovascular system. The heart was grossly enlarged both clinically and by x-ray examination. A murmur and thrill were present which were variously interpreted as indicative of patent ductus arteriosus, interauricular septal defect, or a combination of both lesions. Although the exact nature of the cardiac lesion was uncertain, the presence of some form of congenital heart defect was definite.

4. Skin. Brownish pigmentation of the skin was present, but did not involve the mucous membranes. Again, the parents had noted pigmentation for only three years, but its presence before that time seemed likely.

5. Endocrine system. Gynecomastia was present.

6. Hematopoietic system. The patient showed anemia, leukopenia and neutropenia, and thrombocytopenia (pancytopenia). The cause for the pancytopenia appeared in the bone marrow, which showed hypoplasia of erythropoietic, granulopoietic, and thrombopoietic elements.

The etiology of the underdevelopment of the hematopoietic system could not be attributed to benzol, x-ray, sulfonamides, or any of the other external agents known to produce hypoplasia or aplasia of the marrow in certain instances. The fact that pallor and weakness were already present before the initial dose of sulfadiazine was given for the upper respiratory infections indicates that hypoplastic anemia was already present prior to sulfonamide medication. It is possible, however, that the sulfonamide caused an accentuation of the already existing hypoplasia, which was probably a developmental defect explicable on the same basis as the other developmental defects; i.e., chromosomal abnormalities. The blood picture was typical of idiopathic hypoplastic anemia showing a normochromic normocytic anemia, neutropenia and leukopenia, and thrombocytopenia. The presence of reticulocytosis in certain cases of this disorder, notably those that are congenital in onset, has been noted.<sup>1</sup> Such a disorder would not be expected to respond to treatment with liver or iron, and actually in this patient these medications were without effect on the clinical or hematological status. Splenectomy in hypoplastic anemia is occasionally beneficial, especially in cases in which marked thrombocytopenia is present despite the occurrence of megakaryocytes in the marrow.<sup>1</sup>

The occurrence of hypoplasia or aplasia of the bone marrow in association with other congenital defects designates this case as an example of Fanconi's syndrome. In the absence of a history of any similar disorder in either parent or their families, the case must be regarded as a genetic "sport" due to chance aberration in one or more of the hereditary genes which have to do with development of the bone marrow, skeletal system, heart, etc.

The patient was an only child, and the absence of siblings leaves open the question whether such an occurrence would repeat itself in this family. It is of interest that abnormalities of the thumbs were mentioned in relation to 3 of the 6 reports of Fanconi's syndrome in the literature: the thumbs were absent in a cousin of the cases reported by Hjorth<sup>4</sup>; one thumb was absent in Uehlinger's patient<sup>6</sup>; one thumb was deformed in van Leeuwen's case.<sup>7</sup>

The rationale of splenectomy in congenital hypoplastic anemia has been discussed elsewhere.<sup>1</sup> Splenectomy is of occasional benefit in this disorder, especially in cases in which a hemorrhagic diathesis due to thrombocytopenia is the chief complaint and at the same time the megakaryocytes in the bone marrow are not completely absent. The improvement following removal of the spleen is probably due to removal of the normal inhibitory or regulatory mechanism exerted by the spleen upon the elements produced within the bone marrow. Following splenectomy and probably as a result of elimination of this regulatory factor, the delivery of platelets from the marrow into the peripheral blood may increase sufficiently that the resulting platelet count, although still less than normal, may suffice to prevent the hemorrhagic diathesis. In the present case, thrombocytopenic purpura was a prominent finding and gave rise to a marked bleeding diathesis; but very few megakaryocytes were seen on marrow specimens. Splenectomy was nevertheless undertaken because the patient was going rapidly downhill and it was felt that the operation offered the only chance of improvement. The procedure was followed by a well defined reduction in the necessity for frequent transfusions, though the platelet level did not rise appreciably. The red cell and hemoglobin levels were well maintained and perhaps even favorably influenced. There are no reports of splenectomy in the Fanconi syndrome. In van Leeuwen's patient, who was a 14 year old female child, splenectomy was without effect on the symptoms or signs, but the patient lived for three years after the operation.<sup>7</sup> Splenectomy was also carried out in one of Dacie and Gilpin's patients and was followed by sustained improvement, so that transfusions (which had had to be given regularly preoperatively) were no longer required after the operation. Death here also occurred three years later.<sup>5</sup>

It is of interest to speculate upon the possible relationship of the mother's acute otitis media and mastoiditis, during the third month of pregnancy, to the occurrence of the congenital abnormalities in her child. Recent reports<sup>8,9</sup> have emphasized certain congenital defects following infections early during pregnancy. The most prominent of these concern congenital cataracts in children born of a pregnancy complicated by rubella. Others report eye lesions, heart lesions, deaf mutism, microcephaly, etc., following rubella, chicken pox, mumps, etc. In virtually all reports only virus diseases have been implicated in giving rise to congenital abnormalities, and in all reports the disease affected the mother before the third month of pregnancy. In the present case, it is probable that the infection was completely unrelated to the subsequent abnormalities in the fetus.

#### SUMMARY

A sporadic example of Fanconi syndrome (congenital hypoplastic anemia associated with other congenital defects) is reported.



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# METHOD

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## A SIMPLE APPARATUS AND PROCEDURE FOR PREPARING AND ELECTROPLATING RADIOACTIVE IRON\*

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MARJORIE LAURITSEN, AND M. M. WINTROBE, M.D.

IN THE course of certain studies in this laboratory involving the uptake of radioactive iron by the erythrocytes<sup>1</sup> it became necessary to devise a simple method for the digestion and preparation of the blood samples and to have a relatively simple apparatus for electroplating the  $\text{Fe}^{59}$  preparatory to counting. Despite improvements in apparatus described for this purpose,<sup>2-6</sup> a number of practical disadvantages were encountered when our studies were undertaken.

This paper describes an electroplating apparatus which we believe overcomes some of these disadvantages. It is simple to construct and easy to operate.

Included also in this communication is the procedure we have used for the digestion of the samples and for the isolation of the radioactive iron.

### APPARATUS

A photograph of the electroplating unit is shown in figure 1. The sides are constructed of wood and fiberboard while the frame is of wood. The base supporting the cells is constructed from  $\frac{1}{2}$  inch pressed wood and is treated with chemical resistant paint. A diagram illustrating the electrical connections is shown in figure 2. The cell used for electroplating has been drawn to scale in figure 3. In figure 1 an electroplating cell (A) has been laid on its side to show its construction. Each unit contains eight cells. The bottom half of a  $\frac{1}{4}$  oz. seamless ointment tin,  $1\frac{1}{4}$  inches in diameter, contained in the cell is set on the top half of such a tin which is permanently mounted on the instrument. The portion of the tin which is permanently mounted is connected to the negative pole of the battery. The use of such an ointment tin permits the close approximation of the plated surface to the mica window of the counting tube and provides an air space below the plating surface.

Stirring is accomplished by bubbling cotton-filtered air from a common duct (figure 1, B) through T-tubes and through the solutions, a screw clamp being attached to the inlet of each cell for control. A small pump with a reservoir has given very good service in this laboratory.

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‡ Obtained from Buckeye Stamping Company, Columbus, Ohio.

§ Standard Pressovac Pump, obtained from Gast Manufacturing Corporation, Benton Harbor, Michigan.

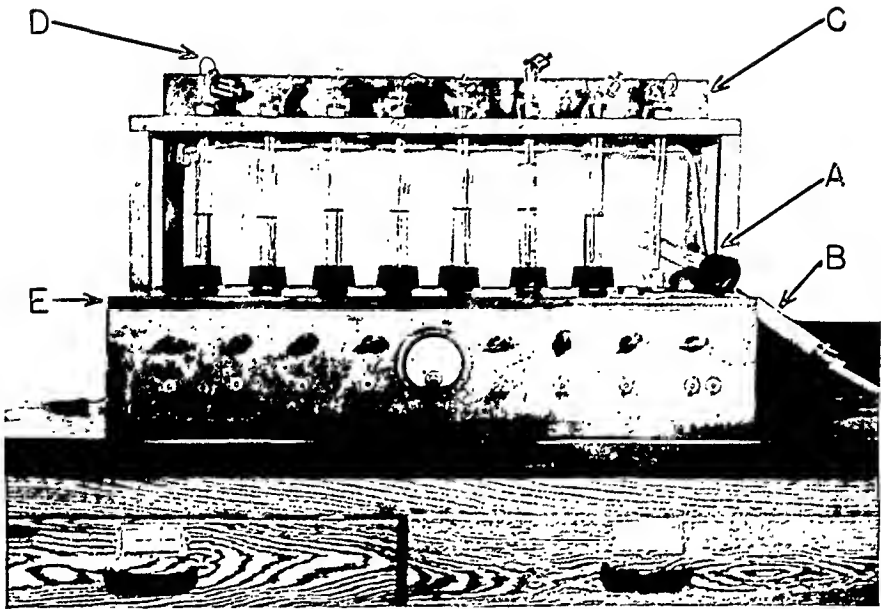


FIG. 1. PHOTOGRAPH OF ELECTROPLATING APPARATUS

The last electroplating cell on the right (A) has been placed on its side for purposes of illustration. At the extreme right of the apparatus (B) lies the tube packed with cotton through which air for the "bubbler" is pumped. The photograph fails to show the sockets in the upper rear panel (C) to which the copper wire (D) of the electrode is connected by means of an electrical prong.

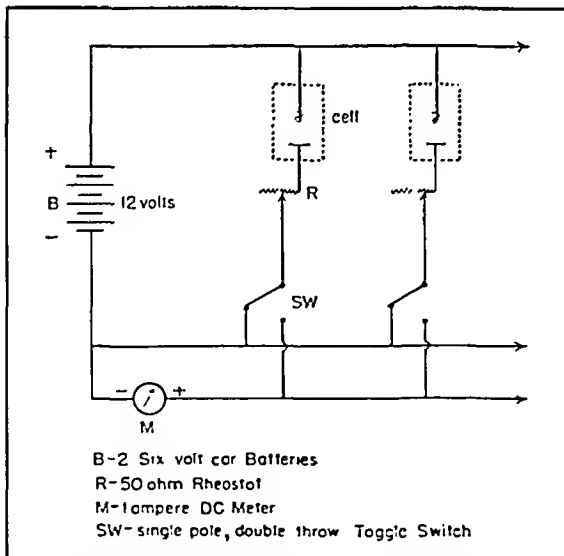


FIG. 2. THE ELECTRICAL CONNECTIONS OF THE ELECTROPLATING APPARATUS

The rubber ring which holds the electroplating cell in place on the ointment tin is prepared as follows. The larger surface of a no. 11 black rubber stopper is centered over the base of an ointment tin of the size used for electroplating, the edge of which is sharpened on a grindstone and which is fastened to a board (fig. 3, h).

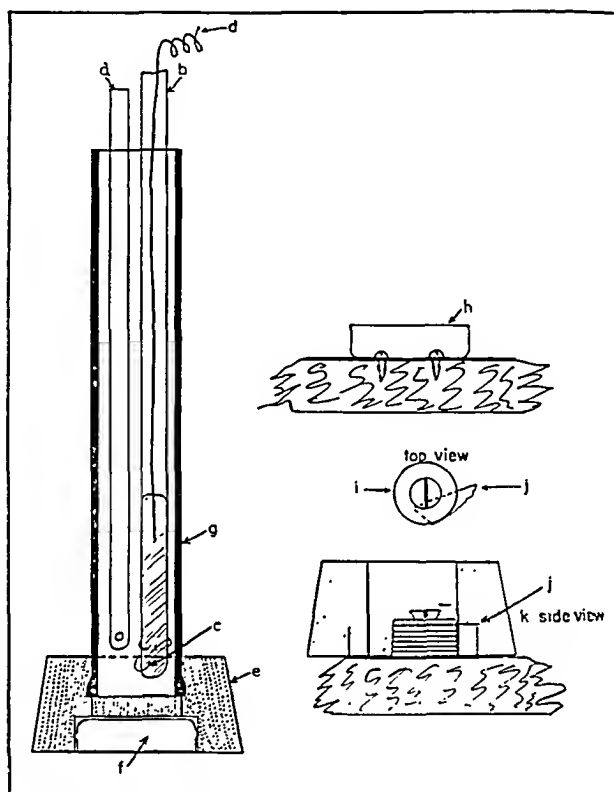


FIG. 3. DIAGRAM, DRAWN TO SCALE, OF THE ELECTROPLATING CELL

In the same figure is shown the technic of preparing the rubber stopper (e) for the cell. a. Glass tube through which air is passed; b. electrode consisting of glass tubing containing mercury into the bottom of which a platinum wire (c) is sealed and in which a copper wire (d) rests. The copper wire is connected to an electrical prong which fits into a socket placed in the upper rear panel of the apparatus (fig. 1, C). The electrode (b) and the air bubbler (a) are fixed in a rubber cork of a size to fit the upper end of the electroplating cell; e. no. 11 rubber stopper; f. ointment tin, bottom half; g. glass tubing, 15 cm. long 2.2 cm. inside, 2.4 cm. outside diam.; h. ointment tin upper half mounted on the wood base of the apparatus (fig. 1, E); i. top view of razor blade (j) fixed in position for cutting rubber stopper; k. side view rubber stopper in place to illustrate method of cutting the ledge.

The stopper is worked down to the board. Since its bore happens to be of the same size as that of the glass tube used for the cell, a Fisher burner altered in the following way is used next as a cork borer. The screen top is removed. With an electric grindstone the edge of the burner is sharpened. The rough edges on the inside are smoothed with a file. This edge is then employed to bore a hole completely through

the cork, starting from the larger surface and working concentrically inside the circle previously cut with the ointment tin. The opening on the larger surface of the stopper is now placed flatly over the unit shown in figure 3, k. This instrument is merely one-fourth of a double-edged razor blade held between washers on a wood screw (fig. 3, i and j). This is used to cut out a ring of rubber which when removed leaves a space in the rubber cork into which the ointment tin can be fitted. The cork is rotated around the edge of the blade, thus cutting a ledge in the cork as shown in the diagram (k, side view). A rubber ring is removed.

The glass tubing is flanged at one end by heating and then pressing down on a rounded wooden object such as the end of a broom handle in order to obtain a perfect circle which when in place in the rubber stopper will fit without permitting leakage. The glass tubing should not be placed directly down to the ointment tin in order that the stopper may exert tension on the ointment tin as well as on the glass tubing. This is illustrated in figure 3.

It is important that the tins be very clean. We remove the shellac with acetone and scrape the plating surface with emery cloth. Failure to do this frequently results in a scaly plate.

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#### DIGESTION AND PREPARATION OF BLOOD SAMPLES OR OTHER BIOLOGICAL SPECIMENS FOR ELECTROPLATING

*Digestion.* The weighed or measured specimen is quantitatively transferred to a 500 ml. Kjeldahl flask. When using blood, about 11 ml. are put into a graduated 15 ml. centrifuge tube containing a sufficient amount of dried double oxalate mixture.<sup>7</sup> From this enough blood is withdrawn to determine the volume of packed red cells and hemoglobin content of the sample. The tube is spun at 3000 rpm. for 15 minutes. The total volume is then easily read. The volume of packed red cells is noted for the purpose of estimating the iron content but for actual calculations the hematocrit reading is used. The plasma is removed and used for other determinations. The red cells are transferred quantitatively to a 500 ml. Kjeldahl flask. Nonradioactive iron is then added to maintain a constant sample iron content of 10 mg. Since the normal mean corpuscular hemoglobin concentration is roughly 33 per cent and the iron content of hemoglobin is 3.39 mg. per gram, then 1 ml. of packed red cells contains approximately 1 mg. of iron. A solution of  $\text{FeCl}_3$  containing 1 mg. of iron per drop is used and enough drops are added to bring the total iron content to 10 mg.

Five ml. of concentrated sulfuric acid, C.P., and 10 ml. of concentrated nitric acid, C.P., are added together with two solid glass beads. This is sufficient to digest the cells from about 15 ml. blood. The specimen is then boiled on a Kjeldahl digestion apparatus\* until the nitrous oxide fumes are driven off or charring occurs. After cooling, approximately 2 ml. of 70 to 72 per cent perchloric acid, C.P., are added and the solution is then boiled to clearness, after which boiling is continued an additional one-half hour. Sometimes a fine white precipitate remains but this disappears when water is added prior to neutralization.

\*Standard model, Precision Scientific Company, Chicago.

The method of separating the iron and the electroplating medium employed are slightly modified from Hahn.<sup>5</sup> A few ml. of water are added to the specimen to prevent sputtering, as well as 2 drops of a 0.5 per cent solution of phenolsulfonphthalein, after which it is neutralized in the flask to an orange color by adding a saturated solution of sodium hydroxide. The neutral solution is then washed quantitatively into a 100 ml. round bottom centrifuge tube and is made just alkaline with the addition of more sodium hydroxide. The tube is allowed to stand at room temperature for two hours or longer to assure complete precipitation of the iron as ferric hydroxide. It should be noted that at this point a crystalline precipitate sometimes forms, particularly if the tube becomes too cool. This can usually be dissolved by the addition of more water; sometimes heating is necessary. This precipitate is then packed by centrifugation and the supernatant liquid is decanted and discarded, after testing it for iron by the qualitative thiocyanate test (a few drops of concentrated HCl, plus 1 drop of 20 per cent KSCN). Conversion to ferric chloride is brought about by the addition of a few drops of concentrated hydrochloric acid. When the precipitate is completely dissolved, the sides of the tube are washed down with distilled water. The solution is now ready for electroplating.

Reduction of the iron is effected by the addition of approximately 70 mg. of ascorbic acid. A drop of 1 per cent phenolphthalein is added as an indicator and concentrated ammonium hydroxide is added drop by drop. The red color of the indicator will appear and disappear after each drop is added until finally the whole solution turns a violet brown with 1 drop. This solution is then washed quantitatively into the plating cell and 2 ml. of saturated sodium citrate are added as a buffer. Water is added to give a final dilution of about 25 ml. The solution generally becomes yellow.

#### OPERATION OF THE APPARATUS

When the bubbler is running, about 400 milliamperes of current are used. This can be measured by switching the meter into the particular cell circuit. The desired amount of current is controlled by the rheostat. The current should be checked after mixing occurs, since it generally rises. It is known that the anode-cathode distance affects the type of plate produced and the speed of plating. We find that alterations in rate of current flow produced by minor variations in the anode-cathode distance are best controlled by means of the rheostat. The anode-cathode distance in our apparatus is about 5 mm.

Without the bubbler about 300 milliamperes is a better level of current since heating will occur if more is used. Our procedure has been to use the bubbler during the day but not overnight. In overnight plating the bubbler is allowed to go for a short time at the beginning of the plating and again for about one-half hour in the morning. This is done to expel any iron that might be present in the glass tubing. An alternative to this procedure is to have separate platinum electrodes without bubblers attached for the overnight plating. Plating is allowed to proceed in every case until a negative thiocyanate test for Fe is obtained. Air should not be bubbled through the solutions too vigorously, as the plates may be oxidized by the excess oxygen. Samples for the iron tests are taken out with a  $1\frac{1}{2}$  inch length of  $\frac{1}{4}$

inch glass tubing constricted at one end and fastened at the other to rubber through which suction is made. When the bubbler is running it is best to take the plates off as soon as the solutions are negative. The plates are numbered on the bottom with wax pencil and are washed with water and 95 per cent ethyl alcohol. After drying the plate is coated with a thin layer of oil (few drops of 1:100 solution of light machine oil in benzene).

The finished plate has a plated surface 2.7 cm. in diameter and 5.7 sq. cm. in area. The total iron plated is 10 mg. and the density of the iron film is therefore 1.75 mg. per sq. cm. Because of self-absorption of radiation in the film itself, the total

TABLE 1.—*Recovery of Radioactive Iron from Blood*

Sample	Fe <sup>59</sup> Added Counts/Minute	No. of Precipitations as Fe(OH) <sub>3</sub>	Recovered Counts/Minute	Per Cent Recovery
1	468*	2	478	102.2
2	468	2	472	100.5
3	468	2	484	103.3
4	468	2	444	94.8
5	468	1	450	96.0
6	468	1	472	100.5
7	468	1	470	100.2
Average.....	468		469	100.2
8	1204†	1	1165	96.8
9	1204	1	1212	100.6
10	1204	1	1182	98.3
11	1204	1	1228	101.8
12	1204	1	1236	102.6
13	1204	1	1204	100.0
Average.....	1204		1204	100.0

\* Average of 8 directly electroplated samples of Fe<sup>59</sup> with a probable error of 4.80 counts/minute.

† Average of 8 directly electroplated samples of Fe<sup>59</sup> with a probable error of 6.01 counts/minute.

iron plated should be kept approximately constant as indicated above. Self-absorption on a film this thin is negligible.<sup>8</sup>

The plate is placed in a slot on a stand which can be raised to a position just below the window of a Geiger tube and there is locked in place. The tube and plate are kept within a lead cylinder 2 inches thick to cut down the background count.

We use a Geiger tube\* with a thin mica window weighing 10 mg. per sq. cm. and having an effective area of 15 sq. cm. Impulses are registered on an electrical impulse register\* after passing through a scale-of-eight counter.\* Each plate is counted long enough to total 4000 to 5000 counts, usually 4 to 12 minutes. The probable statistical error of the count is 1 per cent.

\*Cyclotron Specialties Company, Moraga, California.

## RESULTS

The platings require 2 to 5 hours with the bubbler, whereas without the stirring they are generally complete in 4 to 6 hours.

In table 1 are shown the results of recovery experiments. It will be seen that those counts which had been carried through the entire digestion, precipitation, and electroplating procedures agreed well with the counts obtained when the radioactive iron solutions were added directly to the electroplating cells.

## COMMENT

One unit is capable of plating sixteen samples a day with little attention. In the past few months approximately 1000 samples have been electroplated with no major difficulty. It should be pointed out that the proper pH and sufficient ascorbic acid are necessary to obtain a good plate.

## SUMMARY

A simple, easily constructed apparatus for electroplating radioactive iron has been described by which certain disadvantages of other forms of apparatus have been eliminated. Sixteen samples can be plated each day without difficulty.

The technic employed for preparing the radioactive iron for electroplating has been given.

The writers are indebted to Dr. P. F. Hahn and his staff who permitted one of them (M. L.) to spend several weeks in the Biochemical Laboratory at the Vanderbilt University School of Medicine and generously gave invaluable instruction and advice.

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## EDITORIAL

### IS LEUKEMIA INCREASING?

THE article by Sacks and Seeman in this issue poses a question which has been troubling the interested observer for a number of years: Is leukemia increasing? We seem to see more and more cases of this disease all the time. Granted that this may be largely a "clearing house" reaction, why is it that practitioners in small towns and cities are also seeing more such cases? True, the advent of the technician into the community hospital laboratory and the large numbers of routine blood counts consequently being made undoubtedly uncover many a case which was formerly called anemia or purpura. However, the increasing diagnostic acumen of the modern practitioner with his laboratory aids seems hardly great enough to account for the spectacular jump in the leukemia death rate, as graphically illustrated in Sacks and Seeman's article. It is startling to know that each year since 1940, more than 5000 persons in this country have died of leukemia!

If the incidence of leukemia is indeed increasing what can be the reasons? The cause or causes of leukemia not being known, one hardly dares to speculate on this matter. It is known, however, that radiologists have a definitely higher incidence rate of leukemia than other physicians, and that individuals exposed to radioactivity of various types not infrequently develop the disease. It will be of interest to observe the Japanese survivors of the atomic bomb for future indications of proliferative disease of the white cells. Another etiologic possibility is chemical exposure. In all those hematologic cases in which a careful history of exposure to chemicals is taken, one is struck by the frequency of chemical exposure in the cases of leukemia, particularly in the acute and subacute types. The most common chemicals implicated appear to be benzol, benzol ring drugs, and the arsenicals. The photographer working long hours with developers in a poorly ventilated darkroom, the die and dye workers working respectively with benzol and with aniline dyes, gardeners and foresters for years inhaling arsenical sprays have all figured in well-taken histories of leukemia. To be sure, many other similar cases do not present such stories. But in this chemical age, how do we know which chemical is someday going to start off a leukocytic "spree" in a susceptible individual? The widely used sulfonamides and vitamins; the tars and automobile exhaust gas on the roads; the cosmetics, pimple lotions and under-arm lotions so much in vogue are but a few of the numerous chemicals to which civilized human beings have become increasingly exposed. If chemicals should be found to have a bearing in the causation of leukemia, this may well explain the possible increase in incidence of this disease. This possibility merits careful consideration. Statistical studies of exposure to chemicals in leukemic and nonleukemic cases together with well conceived animal experimentation appear to be indicated.

WILLIAM DAMESHEK, M.D.

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# ABSTRACTS

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## HEMATOPOIETIC TISSUE

OLIVER P. JONES, Ph.D.

ON THE "CONICAL OPENINGS" IN THE WALL OF VENOUS SINUSOIDS AND THEIR RELATION TO THE SO-CALLED ERYTHROGENIC CAPILLARIES IN THE BONE MARROW OF MAN. *E. M. Schleicher*. *Aoat. Rec.* 65: 379-93, 1946.

For many years papers have been published regarding the erythrocytogenic power of endothelium and the nature of the vascular channels in avian and mammalian bone marrow. Several authors (Jordan, H. E., and Johnson, E. P.: *Am. J. Anat.* 56: 71, 1935; McDoomald, J. G.: *Am. J. Anat.* 65: 291, 1939) have discredited the presence of intersinusoidal capillaries as described by Doan et al. (1925). Instead, they have reported stromal or interlipocellular tissue spaces lined by histiocytic elements. Regardless of this controversy, Doan et al., Jordao and Joynson, McDonald, Peabody, Ringoen, Sabin and Miller, etc., have observed and described the openings of these structures directly into the sinusoids. In the present paper, which is based on a study of marrow obtained from a single patient—before and after the intrasternal infusion of 3000 ml. of fresh blood plasma—the author claims that the "conical openings" are closed to the general circulation. This offers no restraint to the entering corpuscles because the author first assumed (without bibliographic reference) that cellular elements pass through the sinusoidal wall after they "have reached a certain physico-chemical state of their colloidal envelopes." Structures originally called intersinusoidal capillaries or stromal and interlipocellular tissue spaces are now designated intraparenchymal sinuses by Schleicher. He has presumed that these structures were produced by loss of fluid from fat cells. However, such minute changes in cellular contour may very well have been due to fixation artifacts—a thing which should have been ruled out.

SOME EFFECTS OF PITUITARY ADRENOTROPIC HORMONE (PATH), EXTRACT OF SUPRARENAL CORTEX, AND COLCHICINE ON THE HAEMATOPOIETIC SYSTEM. *J. M. Yoffey and J. S. Baxter*. *J. Anat.* 80: 132-38, 1946.

One of the hematological problems which has intrigued many workers in the past as well as the present is the nature of the barrier between hematopoietic organs and the peripheral blood. Besides this, there is also the problem of determining the regulatory mechanism which maintains blood cells at relatively constant levels. Since published works on the action of pituitary adrenotropic hormone and extract of suprarenal cortex indicated that lymphocytes were under hormonal rather than nervous control, Yoffey and Baxter undertook certain experiments to confirm these findings. Although their group of test animals was small (26 Wistar rats), the results were so definite that they could be considered as being significant. The administration of pituitary adrenotropic hormone caused a marked diminution of lymphoid tissue of the nodes after 4 weeks and the administration of extract of suprarenal cortex produced the opposite effect to the extent that the nodes were more active than normal. When animals were given both of these substances simultaneously the effects seemed to be neutralized. One of the most interesting observations was the finding that cortical extract produced a lymphopenia in face of hyperactive lymph nodes. Hence, in the absence of degenerative changes, it would seem that the rate with which lymphocytes left the blood was the determining factor in these cases. These authors were unable to produce a significant change in the blood lymphocytes of 2 rabbits after daily subcutaneous injections of cortical extract for 16 and 22 days respectively.

AGE CHANGES IN THE VASCULAR ARCHITECTURE AND CELL CONTENT IN THE SPLEENS OF 100 WISTAR INSTITUTE RATS, INCLUDING COMPARISONS WITH HUMAN MATERIAL. *W. Andrew*. *Am. J. Anat.* 79: 1-74, 1946.

Since very little is known about the age changes of laboratory animals, Andrew undertook a study

of the spleen as a part of a general program sponsored by The Wistar Institute. In addition to studying a pedigreed stock of rats, human spleens from 42 individuals were also studied to see what comparisons could be made. Spleens from the rats were divided into 5 groups according to their age. The most immature animals were 21 days old and the senile group included rats over 726 days old. The human spleens were from unselected autopsy specimens ranging in age from newborn to 92 years. All material was analyzed for: (1) appearance of malpighian follicles, (2) nature of red pulp, (3) amount of pigment, (4) appearance of pigment-containing cells, (5) relative numbers of plasma cells and eosinophils, and (6) the condition of the megakaryocytes. All of the youngest rats had a reticular type of red pulp whereas 77 per cent of the senile group had a sinusoidal type. Reaction centers (germinal) were not present in the most immature group but made their appearance in the group having an age range of 50-150 days. These centers persisted throughout all the remaining groups but were markedly decreased in the senile group. Warren believes that the term "germinal center" is a misnomer and that Hellman's term "reaction center" explains more accurately the function of this structure. Macrophages were not observed in the youngest rats but increased rapidly from 50 days on to 726 days and then decreased somewhat in the senile group. The incidence of megakaryocytes was greatest in the youngest group with a decrease to about one-third in the senile group. Andrew believes certain observations indicate that megakaryocytes may arise by a fusion of smaller cells; however, a few cells were found which supported a hypertrophy of single cells into megakaryocytes. Comparison of the rat spleens with the human spleens showed in general similar things due to age changes. There was a loss of reaction centers, a variable destruction of the pulp architecture, and a change in red pulp from a reticular type to a more sinusoidal type.

CELLULAR GIGANTISM AND PLURIPOLAR MITOSIS IN HUMAN HEMATOPOIESIS. *E. Schwarz*. *Am. J. Anat.* 79: 75-116, 1946.

Although it is customary to divide erythropoietic activity into two main categories, viz., normoblastic and megaloblastic, it has been recognized for some time that giant erythroblasts and erythrocytes exist in normal bone marrow. By using several staining technics on a group of normal and pathologic bone marrows, Schwarz carefully studied the morphology and occurrence of gigantism in hematopoiesis. The clue to the nature of this gigantism was first found by observing giant leukocytes with two normal and independent nuclei. A graded series of these cells in transitional stages could be traced back to a binucleated myeloblast. In the case of erythroblasts, giant forms were found to arise from unicellular erythroblasts which had completed karyokinesis but not cytokinesis. Such cells doubled their cytoplasmic mass, number of centrioles and chromosomes (tetraploid). This process did not interfere with nuclear maturation and hemoglobin formation. While plurinucleated erythroblasts are present in normal bone marrow, they were found to be more numerous in megaloblastic marrows than in hyperplastic normoblastic ones. Since the greatest number of nuclei found was 8, it was suggested that three succeeding divisions apparently exhaust the mitotic activity of an erythroblast (used in the general sense). It is interesting to note that such a condition does not obtain in megakaryocytes, for Japa (*Brit. J. Exper. Path.* 26: 111, 1945) has counted 32 nuclei in these cells. In some cases, after cytokinesis had been suppressed for a while, it appeared that an attempt had been made to resume segmentation. The factor responsible for the suppression of cytokinesis and the production of giant erythroblasts is still unknown.

THE EFFECTS OF IRON, COPPER AND THYROIDINE ON THE ANEMIA INDUCED BY HYPOPHYSECTOMY IN THE ADULT FEMALE RAT. *R. C. Crafts*. *Am. J. Anat.* 79: 267-92, 1946.

Numerous clinical reports as well as the results of experimental hypophysectomy have shown that the pituitary gland has a regulatory effect on erythropoiesis. Since the exact mechanism of the resulting anemia has not been explained satisfactorily, Crafts has attempted to show why this anemia develops in a well controlled group of Long-Evans adult female rats. Hypophysectomy produced a marked drop in the erythrocyte and hemoglobin values after 10 days and then an even greater decrease between 30 and 40 days following the operation. Wright's stained films showed a severe hypochromasia and microcytosis. The bone marrow was definitely hypoplastic. Injections of ferrous sulfate into hypophysectomized rats maintained normal erythrocyte levels for 30 days and hemoglobin values for 20 days before each of these gradually decreased. Intraperitoneal injections of ferrous sulfate and cupric sulfate produced results similar to the administration of iron alone, with the exception that hemoglobin values were maintained for 30 instead of 20 days. Subcutaneous injections of thyroxine maintained the erythrocyte

count at an approximately normal level while the hemoglobin values gradually decreased but not to the level obtained in the untreated hypophysectomized rat. The last group of animals was treated simultaneously with iron, copper, and thyroxin. The erythrocytes were maintained at a normal level but hemoglobin values decreased markedly. At the end of 30 days doses of iron and copper were increased, and after 20 more days it was found that hemoglobin values had risen but not to the normal level. This treatment almost completely prevented hypochromia and microcytosis in addition to producing a hyperplastic bone marrow. These data indicate that the anemia induced by hypophysectomy is perhaps due to a faulty metabolism and that iron may be involved.

LEUKOPENIA AND INFLAMMATION. THE PRESENCE OF A LEUKOPENIC FACTOR IN INFLAMMATORY EXUDATES  
*V. Menkin. Arch. Path. 41: 50-62, 1946.*

For a number of years, Menkin has been interested in the dynamics of inflammation and as a result he focused his attention on the nature of exudates. By using various methods of extraction, fractionation, and purification he was able to isolate from the exudate of an acute inflammatory process substances which would produce cellular damage and necrosis, fever, leukopenia, and leukocytosis. Two substances have been isolated in a relatively pure form and have been called necrosin and pyrexin. The present article reports the results of an investigation to determine the nature of the leukopenic factor. Purified necrosin administered to dogs fails to reduce the absolute number of leukocytes. On the other hand pyrexin not only produced fever but also a marked leukopenia. By subjecting pyrexin to incomplete hydrolysis it was possible to dissociate the leukopenic factor from the pyrogenic factor. Further studies will be necessary to determine whether or not the leukopenic factor is a separate substance or a separate factor in pyrexin.

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# NEWS AND VIEWS

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## PERSONALIA

The First Mexican Congress of Medicine was held in Mexico City August 4 to 10 at the General Hospital. Two of the associate editors of the JOURNAL, Dr. Nathan Rosenthal and Dr. M. M. Wintrobe, were among the invited speakers. They presided over some of the meetings and read papers. Dr. Rosenthal spoke on the "Criteria for the Identification and Classification of the Leukemias," and Dr. Wintrobe spoke on "The Pathogenesis of the Anemia of Infection" and "The Physiopathologic Aspects of the Blood Dyscrasias and Their Diagnosis."

Dr. Oliver P. Jones has been appointed Assistant Dean at the University of Buffalo School of Medicine.

The Ward-Burdick Award of the American Society of Clinical Pathologists was presented to Dr. Philip Levine and Dr. A. S. Wiener at the annual meeting in San Francisco on June 28, 1946. The addresses delivered will appear in the early issues of the American Journal of Clinical Pathology.

Dr. Carl V. Moore has been named Professor of Medicine at the Washington University School of Medicine, St. Louis, Missouri.

Professor Ludwig Hirszfeld, formerly of Warsaw, who has done much of the original work on the heredity of the blood groups and their varying distribution in races, visited the United States for the months of July and August. During his visit Professor Hirszfeld studied the organization of medical schools in America. On his return to Poland he will assume the position of Dean of the Medical School at the University of Wroclaw, formerly known as Breslau.

Dr. Frederick J. Pohle has been promoted to Associate Professor of Medicine at the University of Wisconsin Medical School, Madison, Wisconsin.

Professor R. A. Fisher of Cambridge University, England, delivered a lecture on "The Rhesus Factor, a Study in Scientific Method," on August 15 at the Britton Auditorium, Connecticut Agricultural Experiment Station, New Haven, Connecticut. In this lecture Professor Fisher presented his views on a series of closely linked genes to explain the heredity of the Rh factor.

Dr. Lloyd F. Craver, New York, spoke on "Tumors of the Lymphatic System and Leukemia" at the nineteenth graduate fortnight of the New York Academy of Medicine in October.

Dr. José A. Silva, a Rockefeller Foundation Fellow in Hematology from the Philippines, and Dr. Lucio Penna de Carvalho Lima of São Paulo, Brazil, have joined Dr. M. M. Wintrobe's laboratory at the University of Utah.

The International Hematology and Rh Conference was held in Dallas, Texas, on November 15 and 16, 1946. Upon completion of the meetings the entire conference moved to Mexico City, where it was affiliated with the Second Mexican Blood Transfusion Congress. Meetings of both groups were continued from November 17 to 23, 1946. Dr. Joseph M. Hill, Director of the William Buchanan Blood, Plasma and Serum Center of the Baylor Hospital, and Dr. Sol Haberman of the same institution were the President and Secretary respectively of the Texas conference. Dr. Eduardo Uribe Guerola was President of the Second Mexican Transfusion Congress, and Dr. Alfonso Velez Orozco, Secretary. The speakers at both congresses were Dr. Philip Levine, Dr. R. R. Race of London, Dr. William Dameshek, Dr. Ernest Witebsky, Dr. I. Davidsohn, Dr. Louis K. Diamond, Dr. Ignacio González-Guzmán, Dr. Mario Salazar Mallen, Dr. Bruce Chown, Dr. J. M. Hill, and Dr. Sol Haberman.

Dr. Harry Wallerstein showed a film on complete substitution transfusion in the treatment of acute hemolytic disease of the newborn.

Lively discussions relating particularly to immunohematology and the Rh factor took place and will be reported upon in a subsequent issue of the Journal.

First steps were taken towards the formation of an International Society for the Study of the Blood and preliminary discussions took place relating to plans for another congress.

Surgeon General Thomas Parran has appointed a panel called the Hematologic Study Section of the National Institute of Health. Dr. Kenneth Endicott of the National Institute of Health at Bethesda is the Executive Secretary of this panel. One of the functions of the panel will be to make recommendations for grants-in-aid to investigators doing hematological investigations.

#### MISCELLANEOUS

The Department of Hematology of the Mount Sinai Hospital, New York, has grown considerably during the past year, and a branch of Experimental Hematology has recently been inaugurated with Dr. A. L. Copley appointed to this branch.

A Fellowship has been established in the Department of Medicine and Obstetrics of the University of Maryland School of Medicine and College of Physicians and Surgeons, Baltimore, for the study of problems relating to the Rh factor. This Fellowship will carry a stipend of \$2,500 a year. It is sponsored by the obstetric and gynecologic section of the Baltimore City Medical Society. Work is to be done in a special laboratory established for this purpose at the University of Maryland School of Medicine. Interested applicants should communicate with the director of the Baltimore Rh Typing Laboratory, Dr. Milton S. Sacks, University of Maryland.

land School of Medicine and College of Physicians and Surgeons, Baltimore 1.  
(From the Journal of the American Medical Association, June 29, 1946.)

Dr. Charles A. Doan and his staff gave a postgraduate course in hematology primarily for the members of the American College of Physicians in Columbus during October.

The Sandoz Chemical Works has given a grant of \$1,000 a year to F. R. Goetzl, Permanente Foundation, Oakland, California, for studies on blood coagulation, according to Science.

A film on blood prepared by the Armour Company under the direction of the American College of Surgeons has been completed. It was shown at the A.M.A. meeting and will be lent to hematologists throughout the country. Dr. Florian E. Schmidt, Director of Medical Motion Pictures of the Armour Laboratories, should be contacted for further information.

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# BOOK REVIEWS

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*The Principles of Heredity.* By LAURENCE H. SNYDER. D. C. Heath and Company, Boston. 3rd edition. Pp. 450. \$3.75.

Of the many books on genetics, this text by Snyder may be highly recommended to physicians. The author has been actively interested for many years in all aspects of human heredity. Although the first part of the book deals with the theoretical principles of genetics, numerous illustrative cases are presented from human material. In the first half of the book such fundamental principles as Mendelian laws, determination of sex, lethal factors, linkage groups and sex-linked factors, multiple alleles, and selection and inbreeding, are presented concisely and yet sufficiently comprehensively for both students of genetics and for physicians. The genetic story of the blood groups, the M N, and Rh factors are presented in the chapter on multiple alleles. It is probable that in the future the British theory of closely linked genes for the heredity of the Rh-Hr system will be accepted. This will necessitate a change in the present terminology which does not take into account the three Hr factors. Fortunately, only passing reference is made to the role of the Rh factor and feeble-mindedness since the correlation is a loose one.

Of particular interest to physicians and research workers is the chapter "How Genes Act" which deals with the fundamental work on the genetic basis of enzymes in determination of color differences in flowers and disturbances in metabolism in man and animals. A brief description is given of the important work of Beadle on *Neurospora* with its implications in growth promoting substances and more specifically in such errors of metabolism as cystinuria and alkaptonuria.

Other chapters, such as "The Mutant Gene in Man," "Eugenics," and "Analysis of Human Histories," will appeal to the physician because they deal mainly with clinical material. Here he will find maps of human chromosomes, a listing of many genetic properties, normal and pathologic, and an authoritative statement concerning their mode of heredity so far as it is known at present. Another attractive feature of this excellent book is the inclusion of problems and questions at the end of each of the twenty-nine chapters.

*Stitt's Diagnosis, Prevention, and Treatment of Tropical Disease.* By RICHARD P. STRONG. The Blakiston Company, Philadelphia. 7th ed. (1944, reprinted) 1945. Pp. 1750. \$15.

The importance of the tropical diseases was at its peak during the war years, as attested by the six editions or printings of this standard work from January 1942 to February 1945. Admiral Stitt published the first edition in 1914 and in 1941 turned it over to Dr. Richard P. Strong for revision. The resulting work is a handsome volume of some 1750 pages replete with illustrations and with an excellent bibliography at the end of each chapter. There are excellent descriptions of the various diseases with full discussions of pathogenesis, pathology, prophylaxis, and treatment. The various concepts relating to the mechanisms involved in the sudden hemolysis of blackwater fever are discussed at length. The anemias of tropical countries are authoritatively discussed in the section on problems of medical practice in the tropics. The appendix has large sections dealing with an index of clinical diagnosis, laboratory diagnosis, and tropical hygiene. It is easy to see that with this large volume alone, an intelligent physician could practice medicine in the tropics almost unassisted. Since the tropical diseases have, in some measure, come to us in this country with the return of the veterans, it behooves all practicing physicians to keep in touch with this important subject, and there is no better way to do it than with this book.

*Contribución de la Citología en el Diagnóstico de las Afecciones de la Sangre y de los Órganos Hematopoyéticos.* By PEDRO PASEYRO. Thesis, Montevideo. Editorial Medico-Quirurgica, Montevideo, 1946.

The author describes his experiences with puncture of lymph nodes, spleen, bone marrow, and liver and lists several hundred cases in which "adenograms, splenograms, myelograms, and hepatograms" were performed.

Punctures of the lymph nodes, spleen, and liver are still done only occasionally in this country although the sternal puncture has steadily gained in popularity. Lymph node punctures in particular are readily performed and often yield interesting and at times important information. Paseyro presents good photomicrographs, for example, of neoplastic metastasis, tuberculosis, and Hodgkin's disease, and cites the ease and importance of studying the progressive phases in a lymphomatous process. As obtained with the puncture technic, the cells are larger and more subject to intimate histologic study. Punctures of the spleen and liver are becoming more frequent, but are preferably performed in or close to an operating room in case of a possible accident or hemorrhage. The author cites numerous cases in which these tests were important in settling a diagnosis.

The book is well set up and written and contains many good photomicrographs and other illustrations. The Latin Americans and the French have devoted much attention to this field, and we in this country might profit by their teachings.

*Moderne Eisentherapie.* By RUDOLF STODTMEISTER AND PETER BÜCHMANN. Pp. 120, \$3.75. Published and distributed by authority of the Alien Property Custodian, J. W. Edwards, Publisher, 1944. Lithoprinted from original publication of Wissenschaftliche Verlagsgesellschaft, M.b.H. Stuttgart, Germany, 1943.

This little volume, which was published in Germany during the war, has been lithoprinted in this country by authority of the Alien Property Custodian. It is an excellent review of the whole status of iron in medicine, with an historical account of iron therapy, the iron content of various organs, the serum iron, iron absorption, and the use of various types of iron compounds in therapy with their indications in various conditions. Particular attention is paid to iron therapy, especially in the iron deficiency states. The book contains most of the available material on iron and can be recommended for review purposes. Only a few citations of the literature are made. The authors admit their indebtedness to Professor Ludwig Heilmeyer of Jena.

*L'Anémie infectieuse.* By G. HEMMELER. Benno Schwabe & Co., Basel, Switzerland. Pp. 76 5 francs

This little paper-covered book is concerned with the anemia of infection in which there has been relatively little interest although a few authoritative articles, notably from Wintrobe's clinic, have recently appeared. The object of the present work is to study the frequency of infectious anemia, the changes in blood and bone marrow, the pathogenic mechanisms, and finally therapy. It was found that most febrile diseases resulted in anemia, most pronounced in cases with the greatest elevations in temperature, sedimentation rate, and leukocytosis. A series of 25 cases is presented in which careful studies of the blood morphology and the sternal bone marrow were made. These showed a normochromic hypoplastic type of anemia due to a lack of erythroblastic maturation. Serum iron studies gave low values indicating that the anemia was "toxic" in type with a direct disturbance in erythropoiesis. It was found that transfusions of blood represent the only valuable method of therapy of the anemia.

This monograph can be recommended as a clear straightforward account of most of our available knowledge of the anemia in infection and should help to fill the gap which the author says exists in the description of this common and therefore important field. The author probably did not have access to the comprehensive study by Dr. Gullt Lindh Muller of the blood in tuberculosis (*The Commonwealth Fund*, 1943).

*Peripheral Vascular Diseases.* By EDGAR V. ALLEN, B.S., M.A., M.D., M.S. in Medicine, F.A.C.P., Division of Medicine, Mayo Clinic, Assoc. Prof. Medicine, Mayo Foundation, Graduate School, Univ. Minnesota, Diplomate of the American Board of Internal Medicine, and NELSON W. BARKER, B.A., M.D., M.S. in Medicine, F.A.C.P., Division of Medicine, Mayo Clinic, Assoc. Prof. Medicine, Mayo Foundation, Graduate School, Univ. Minnesota, Diplomate of the American Board of Internal Medicine, and EDGAR A. HINES, JR., M.D., B.S., M.A., M.S. in Medicine, F.A.C.P., Division of Medicine, Mayo Clinic, Assoc. Prof. Medicine, Mayo Foundation, Graduate School, Univ. Minnesota, with Associates in the Mayo Clinic and Mayo Foundation. Pp. 871, with 386 illustrations, - in color Philadelphia and London W. B. Saunders Company, 1946. \$10.00

*Peripheral Vascular Diseases* by Allen, Barker, and Hines is dedicated to the late George E. Brown and

is written by his former associates and students at the Mayo Clinic. Over twenty years ago Brown began the establishment of a Section in Peripheral Vascular Diseases on the Medical Service of the Mayo Clinic, and this book, in large part, represents the clinical experiences of the group. Except for a small surgical section, the book is written by internists and embraces the large picture of peripheral vascular diseases. Omitted from consideration are arterial hypertension and vascular diseases of the central nervous system. The first six chapters are concerned with definition of terms, the anatomy of the peripheral blood vessels, and the general principles of diagnosis, including special methods of investigation. In the following chapters the principal peripheral vascular diseases are taken up sequentially. In the discussion of each disease the historical data are first considered, followed by the pathology, pathological physiology, etiology, diagnosis, clinical course, prognosis, and treatment. Four chapters are devoted to the consideration of diseases of the veins. At the end of the book one chapter is devoted to special medical technics in treatment and one to surgical treatment of certain peripheral vascular diseases.

This is an excellent book and is a great contribution to medical literature. Physicians working in the field of peripheral vascular diseases will find it very useful and, in addition, the general practitioner and general internist will find it most helpful for reference since the material is well arranged. There is an excellent bibliography with each chapter. A large amount of valuable statistical data from rather complete studies is presented for all the important diseases, and the enormousness of the careful experimental and clinical diagnostic work done on patients by these authors can best be appreciated by those who have worked in this field. For the student beginning an intensive study of peripheral vascular diseases the book offers the historical aspect of these subjects and presents him with a number of unsolved problems for investigation brought out by a presentation of the pathology and physiology as it is known today. The sections devoted to clinical diagnosis and special methods of investigation are particularly good. The book is well illustrated.

The somewhat tiresome style of writing, probably unavoidable in a book written in sections by various authors, is compensated by the completeness which makes the book valuable to medical students as well as physicians.

While the book can be used profitably by surgeons both as a reference book and for fundamental education, it is not designed for them. Only fifty-nine pages of the eight hundred seventy-one are specifically devoted to surgical technic. In these are considered amputation for occlusive arterial diseases, the surgical treatment of traumatic arteriovenous aneurysms, and the treatment of varicose veins. For the sake of completeness the reviewer would like to have seen a larger surgical section, particularly more material on the surgical treatment of aneurysms. The whole subject of arterial injuries and arterial anastomosis which has been such a problem in military surgery is not discussed. The authors' views on the treatment of thrombophlebitis and the whole question of prevention of pulmonary embolism will undoubtedly interest practicing surgeons, since they are fundamentally opposed to the operative approach.

# BLOOD

## *The Journal of Hematology*

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### DIETARY FACTORS CONCERNED IN ERYTHROPOIESIS

By GEORGE E. CARTWRIGHT, M.D.

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#### I. INTRODUCTION

KNOWLEDGE concerning the formation of the erythrocyte is far less widespread than that concerning its destruction. The breakdown through the various bile pigments has been extensively studied and several of the intermediary compounds have been isolated and identified. Although hemin has been synthesized *in vitro* by Fischer and Zeile<sup>1</sup> there is little evidence to indicate that such steps take place *in vivo*. The intermediary compounds have not been isolated, and this approach has not been productive. However, it is known that the absence of certain dietary essentials retards erythrocyte formation and the addition of these essentials to the diet accelerates it. It seems hopeful that a study of the dietary factors concerned in the formation of the erythrocyte will define the buildingstones and in time allow them to be pieced together so as to afford an understanding of the subject. It might be expected that as each factor is clearly defined, relationships will be discovered and the processes of hemoglobin formation and erythropoiesis will unfold. It is the purpose of this review to describe those dietary factors necessary for erythropoiesis which are now recognized and to discuss the present knowledge concerning them.

The known factors concerned in the formation of the red cell may be divided into three groups: vitamins, amino acids, and minerals. It might be supposed that these substances could be divided into those concerned with the formation of the stroma of the red cell and those concerned directly with the formation of hemoglobin. At the present time it is impossible to make this division, and it will be necessary to consider the formation of the erythrocyte and hemoglobin as one process, erythropoiesis. The term "hemoglobin formation" has been used here loosely, as in the literature, to refer to erythropoiesis.

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Because of the enormous volume of literature on the subject much of it has had to be omitted. Much of the earlier work in which complex diets and crude extracts were used is inconclusive and for this reason has been omitted. Data regarding the type of anemia produced have had to be reviewed critically. Although much of the nutritional work has been carefully done detailed hematologic studies have been neglected. Studies of the cell size and of the concentration of hemoglobin in the cells have many times not been made. The terms "hypochromic," "normochromic," and "hyperchromic" have been used in the literature loosely. These terms are employed in this review only when referring to studies in which the mean corpuscular hemoglobin concentration was calculated. In general, only that work which is clearly defined has been reviewed.

The nutritional, chemical, and physiological approach to the formation of red blood cells is relatively new and views are ever changing. With each change certain concepts are proved false, new ones are added, and others are more firmly established. This is both natural and desirable. It is a dynamic field for investigation.

## II. VITAMINS

It has long been suspected that vitamins play a role in red blood cell formation but it was not until "synthetic" diets were available that a specific vitamin deficiency could be studied. It has now been definitely shown that riboflavin, nicotinic acid, pyridoxine, and the various "folic acids" are important for red blood cell formation in at least one species each. The role of ascorbic acid, pantothenic acid, choline, and biotin in erythropoiesis is not clearly established and there is no substantial evidence that thiamine, *p*-aminobenzoic acid, or inositol is concerned.

A. Riboflavin.—The role of riboflavin in blood regeneration in dogs on a purified ration has been carefully studied by Spector, Maass, Michaud, Elvehjem, and Hart.<sup>2</sup> A mild anemia developed on the purified ration without riboflavin and a severe anemia was readily induced with slight bleeding. In spite of cessation of phlebotomy the dogs were unable to recover from the anemia. The average weekly production of hemoglobin was only 2 to 6 grams. The animals showed good hemoglobin regeneration and recovered rapidly from the anemia when 30 milligrams per kilo of body weight of riboflavin was administered per day. Only slight and variable hemoglobin production was observed below a level of 7  $\gamma$  per kilo of body weight per day in growing dogs. The adult dog was able to show good hemoglobin regeneration at a level of 15  $\gamma$  per kilo daily. During the depletion period the anemia produced was of the microcytic hypochromic type. If riboflavin was given but phlebotomy was carried out, a decrease in hemoglobin, hematocrit, and erythrocyte count took place but the anemia was normocytic hypochromic in type. From this the authors conclude that riboflavin plays a role in determining the size of new red cells.

Wintrobe, Bushke, Follis, and Humphreys<sup>3</sup> have reported the development of anemia in two riboflavin-deficient pigs which survived a long time. The anemia

was moderate, gradually progressive, and normocytic. Leukopenia was not marked although the leukocyte count at the time of death was low as compared with the initial values. Demyelination of the brachial and sciatic nerves was found in one animal. Spinal cord changes were not noted.

A moderate impairment in the rate of red blood cell and hemoglobin regeneration in the rat as a result of riboflavin deficiency has been demonstrated. Anemia was noted in a few riboflavin-deficient rats not subjected to hemorrhage.<sup>4</sup> Waisman<sup>5</sup> has reported that monkeys maintained on a highly purified diet consisting of purified casein, sucrose, salts, corn oil, cod liver oil, and adequate quantities of pure vitamins except riboflavin, together with a folic acid concentrate, develop an unmistakable anemia. Maximal anemia developed in 59 to 108 days. The red cell counts fell from a level of 5 to 6 million down to 2 to 3 million and the hemoglobin from 13 to as low as 4 grams. The reduced red cell count usually preceded the decrease in hemoglobin. There was also a definite lowering of the white blood cell count. In a more recent paper from the same laboratory<sup>6</sup> the presence of anemia in monkeys on a riboflavin-deficient diet was confirmed. Determinations of the cell size and corpuscular hemoglobin concentration were not done. Upon administration of riboflavin there was a definite increase in hemoglobin, red cell and white cell count in the blood.

A relationship between riboflavin and erythropoiesis was suggested but not definitely proved in the earlier literature. Johnstone and Reed<sup>7</sup> reported that monkeys maintained on riboflavin-deficient diets developed a macrocytic anemia, hypochlorhydria, and leukopenia. Their diet, however, was deficient in all other members of the B complex except thiamine. Hemorrhagic anemia in rabbits was reported as responding in part to riboflavin administration.<sup>8</sup> Earlier work in the dog failed to demonstrate an anemia but myelin degeneration of peripheral nerves and posterior columns of the spinal cord was reported.<sup>9</sup> Sebrell and Onstott<sup>10</sup> reported that dogs maintained on a diet of polished rice, casein, sucrose, cornstarch, cottonseed oil, cod liver oil, and salt mixture developed a "hypochromic" anemia with hemoglobin values ranging between 7 and 13 grams per cent. The bone marrow was reported as being largely fatty. However, there was no constant and material improvement in the blood picture following the administration of large doses of riboflavin, and it is quite likely that the anemia observed was due to pyridoxine deficiency rather than to riboflavin deficiency. In 1933 Miller and Rhoads<sup>11</sup> reported that dogs maintained on a modified Goldberger "black tongue"-producing diet developed glossitis, stomatitis, diarrhea, a definite macrocytic anemia, and a hyperplastic megaloblastic bone marrow not unlike that seen in sprue and pernicious anemia. Since riboflavin was not given to these animals to test the response of the blood it was not demonstrated that the changes were due specifically to riboflavin deficiency. These animals were also deficient in other vitamins. More recent work using purified diets has failed to confirm the presence of a macrocytic anemia. György, Robscheit-Robbins, and Whipple<sup>12</sup> found that the administration of a synthetic riboflavin to standardized anemic dog, produced a definite increase in hemoglobin production above the basal level. Potter, Axelrod,

and Elvehjem<sup>13</sup> found that dogs on a highly purified diet deficient only in riboflavin developed a mild anemia with hemoglobin values ranging between 9.9 and 12.3 Gm. per cent.

There is no substantial evidence that riboflavin deficiency in human beings results in anemia. Gobell<sup>14</sup> obtained increases in the red blood cell count of premature infants by the administration of nicotinamide and riboflavin. Sebrell and Butler<sup>15</sup> maintained 18 patients for 335 days on a riboflavin-deficient diet and found no significant change in the hemoglobin or red and white blood cell counts. Moore et al.<sup>16</sup> found that the hypochromic anemia occurring in patients with ariboflavinosis responded favorably and completely to iron therapy alone. Since myelin degeneration of the peripheral nerves and posterior columns of the spinal cord occurs in riboflavin-deficient dogs it was suggested that this vitamin might play a role in the etiology of subacute combined degeneration of the cord. However, the absorption and excretion of riboflavin has been shown to be normal in patients with pernicious anemia<sup>17</sup> and therapy with this vitamin is ineffective. That riboflavin is not the extrinsic factor has been conclusively demonstrated.<sup>18-20</sup>

TABLE 1.—*Hematological Findings in Chronic Nicotinic Acid Deficiency in Dogs. Modified After Handler and Featherston<sup>22</sup>*

State	R.B.C. Millions per cu mm.	Hemo- globin Gm. per 100 cc.	Hema- tocrit per cent	Mean Corpus- cular Volume cu. micra	Mean Corpus- cular Hemo- globin %	Mean Corpus- cular Hemo- globin conc. per cent.	Reticu- locytes per cent
Normal	6.9	15.2	45.5	66	22	33	<1
Anemic. ....	2.8	6.8	25.1	89	24	27	<1
Normal ....	7.2	16.6	51.4	71	23	32	<1
Anemic.....	2.3	5.5	16.9	74	24	32	<1

Klein and Kohn<sup>21</sup> have shown that the synthesis of flavinadenine dinucleotide from riboflavin takes place in human blood cells both *in vitro* and *in vivo*. It seems possible that this enzyme may be related to erythropoiesis or hemoglobin synthesis. It is known that d-amino acids are deaminized by an enzyme which contains the flavinadenine nucleotide, and Spector et al.<sup>2</sup> have suggested that this vitamin may be concerned in the metabolism and arrangement of the amino acids of globin.

B. Nicotinic Acid.—Anemia due to nicotinic acid deficiency was obscure and indefinite until Handler and Featherston<sup>22</sup> demonstrated that the parenteral administration of physiological saline solution to dogs with acute black tongue resulted in alleviation of the existing hemoconcentration and was associated with the appearance of severe anemia (table 1). Determination of total blood volume and total circulating hemoglobin revealed a reduction of 86 per cent in the total circulating hemoglobin. Thus, since the total blood volume was greatly reduced the anemia was actually more severe than it appeared to be. The total hemoglobin content of the normal dogs averaged 284 grams while the nicotinic acid-deficient animals possessed an average of only 39 grams of hemoglobin. The anemia fell into

no definite groups in respect to cell size. In one group it was macrocytic with an increase in the mean corpuscular volume from 66 to 89 cu. microns. The mean corpuscular hemoglobin concentration declined in proportion to the increase in cell size. In the second group the anemia was normocytic and normochromic. There were no instances of microcytosis. Gastric analyses were performed on 14 dogs and in no case was there any apparent impairment of acid secretory function. There was no reticulocytosis accompanying the anemia and the total serum bilirubin was not elevated. The anemia failed to respond to the administration of iron, rotein, glucose, hemoglobin, antipernicious anemia factor, xanthopterin, and obalt. Following the administration of nicotinic acid or nicotinamide there was an immediate reticulocyte response which reached a maximum of 15 to 30 per cent after 3 to 4 days and subsided in about 10 days. The red cell counts, hemoglobin, and volume of packed red cells continued to rise following the reticulocytosis and reached normal in 30 to 40 days. The red cells returned to their original size in every instance.

Histological examination of the tissues of one dog was made and revealed hemosiderosis of the spleen. The femur marrow contained hemosiderin granules and in addition appeared "exhausted." The cells of the leukocytic series were reduced in number and there was little erythropoietic tissue. Erythropoiesis appeared to have stopped at the erythroblast level. These authors postulated that since immature nucleated erythrocytes respire, they probably use pyridine nucleotides in this respiration. As the supply of nicotinic acid diminishes, anemia develops owing to lack of cozymase in the earliest stages of cell development.

Thus, in dogs, nicotinic acid deficiency results in a severe anemia which is either macrocytic normochromic or normocytic normochromic and is accompanied by a hypoplastic bone marrow. The anemia responds to nicotinic acid therapy.

Nutritional panmyelophthisis in rats<sup>23, 24</sup> has been reported as being prevented by nicotinic acid. This has not been confirmed.

The association of anemia with pellagra has long been known. Anemia is reported in various series to be present in 44,<sup>25</sup> 63,<sup>26</sup> and 84<sup>27</sup> per cent of the cases. It is variable in nature. Huck,<sup>25</sup> Boggs and Padget,<sup>29</sup> and Mallow and Klein<sup>30</sup> observed a "secondary" type of anemia in all of their cases except one. Turner<sup>25</sup> in a careful study of 22 anemic pellagrins found that the anemia was either normocytic or microcytic and in no instance was it macrocytic in type. Thirty-four per cent had erythrocytes with corpuscular hemoglobin concentration less than normal, while in 66 per cent the concentration was within normal range. Spies and Rhinn<sup>26</sup> in a study of 30 severe "alcoholic" pellagrins found that in 75 per cent there was a definite increase in the volume of the red blood cells and a color index of one or above. In the other 25 per cent there was an anemia which was characterized by a decrease in the red blood cell volume and color index. Moore, Vilter, Annich, and Spies<sup>31</sup> have reported the occurrence of macrocytic anemia in 56 patients who had existed for years on diets inadequate in animal protein and in the vitamins of the B complex. Most of the patients had clinical evidence of pellagra, ariboflavinosis, or beriberi. Niacin given in conjunction with 7 other known synthetic B vitamins did not affect the erythropoietic equilibrium. Moore,

Minnich, Vilter, and Spies<sup>16</sup> in a study of hypochromic anemia in 32 patients with pellagra and other vitamin deficiencies reported that iron therapy alone produced a satisfactory reticulocyte response and rate of hemoglobin regeneration. Thus, there is no evidence that the anemia associated with pellagra is due to nicotinic acid deficiency. Proof that such an anemia exists in man is lacking.

Gastric achlorhydria occurs in approximately 80 per cent of the patients with pellagra.<sup>27</sup> However, it has been demonstrated that the intrinsic factor is present in the gastric secretions from pellagrins in an amount adequate to form the anti-anemic substance.<sup>32</sup> That nicotinic acid is not the extrinsic factor of Castle has also been adequately demonstrated.<sup>20, 33</sup>

It is now generally agreed that an increased excretion of porphyrin in the urine is not an essential feature of pellagra and when porphyrinuria appears it is most likely due to some alteration of liver function.<sup>34, 35</sup>

C. Pyridoxine.—The development of severe anemia has been adequately demonstrated to result from pyridoxine deficiency in dogs<sup>36-41</sup> and swine.<sup>42-46</sup> A mild anemia develops in pyridoxine-deficient chicks<sup>47</sup> and in this species the anemia is accompanied by a decreased clotting time, hyperprothrombinemia, and small spleens. The earlier reports on pyridoxine-deficient rats either failed to reveal the presence of anemia or at most only a slight anemia was noted.<sup>48</sup> Kornberg, Tabor, and Sebrell<sup>49</sup> have recently studied blood regeneration in pyridoxine-deficient rats in detail. A moderately severe anemia was found in 18 per cent. Granulocytopenia was present in 4 of these. In 15 per cent a mild anemia was noted. No anemia was found in 67 per cent. A latent erythropoietic inadequacy indicated by an impairment in the rate of red blood cell regeneration after hemorrhage was demonstrated in all pyridoxine-deficient rats.

Morphologically the anemia in dogs and swine is microcytic and slightly hypochromic. In dogs there is a reduction of the mean corpuscular volume from approximately 70 to 48 cubic microns. The mean corpuscular hemoglobin concentration is slightly reduced. In swine the mean corpuscular volume is reduced to an average of 38 cubic microns from an average of 58 and the mean corpuscular hemoglobin concentration changes from 33 per cent to 29 per cent. The anemia is severe in both species, the hemoglobin falling as low as 1.4 grams per 100 cc. of blood. In pigs a volume of packed red cells as low as 9 cc. per 100 cc. of blood has been reported. A significant anemia develops in either species within two months. In pigs, as anemia develops anisocytosis becomes marked but poikilocytosis is rare. The "Price-Jones" curve of distribution of the red cell diameters is "shifted to the left" and the mean diameter is reduced. Large polychromatic red corpuscles make their appearance but are outnumbered by the microcytes. An irregular reticulocytosis as great as 10 to 12 per cent may appear. A large number of red corpuscles are seen to contain a single, round and moderately large, blue-staining granule resembling a nuclear particle. Normoblasts may be increased to as many as 4 and even 8 per 100 leukocytes.

The bone marrow in pyridoxine-deficient swine is hyperplastic.<sup>44</sup> The femoral marrow is cellular rather than fatty and there is a definite increase in nucleated red

blood cells as well as in undifferentiated "blast" cells. Following therapy with crystalline pyridoxine the marrow becomes normoblastic. Information concerning the marrow of pyridoxine-deficient dogs is scant. Fouts et al.<sup>36</sup> made such an examination in only one animal, and in this animal the marrow was red and hyperplastic and consisted chiefly of normoblasts. In the one animal studied after therapy the bone marrow was normal.

Hemosiderosis of the spleen, liver, and bone marrow has been reported in swine.<sup>44</sup> There is a great accumulation of hemosiderin in the pulp of the spleen which is both intra- and extra-cellular but the granules are conspicuously absent from the malpighian bodies. Following therapy the hemosiderosis of the liver and bone marrow disappears and that in the splenic pulp diminishes. By restricting the intake of iron the hemosiderosis can be entirely prevented.<sup>46</sup> Hemosiderosis in pyridoxine-deficient dogs has not been reported.

Ataxia and convulsions have been reported to develop in both dogs<sup>40</sup> and swine<sup>42-45</sup> and pathologically the nervous system shows interesting changes. Degeneration takes place in the peripheral nerves, the spinal ganglia, the posterior roots, and the dorsal funiculi of the spinal cord. It would seem that pyridoxine, like the antipernicious anemia substance and copper, is essential to the integrity of both the nervous and hematopoietic systems.

There is no evidence for the presence of a hemolytic element in pyridoxine deficiency anemia. McKibbin, Shaefer, Frost, and Elvehjem<sup>41</sup> reported that the plasma bilirubin level was normal in dogs deficient in pyridoxin. Cartwright, Wintrobe, and Humphreys<sup>46</sup> studied serum bilirubin, per cent reticulocytes in the blood, icterus index, quantitative urobilinogen excretion in the stool and urine, and urinary excretion of porphyrin in swine and compared this anemia with that induced by phenylhydrazine. They could find nothing to indicate that an increased rate of hemolysis occurs in pyridoxine deficiency.

Elevated plasma iron levels in dogs were reported by Fouts et al.<sup>39</sup> and confirmed by McKibbin et al.<sup>41</sup> The serum iron which is normally around 100  $\gamma$  per cent rises to the extremely high values of 300 to 500  $\gamma$  per cent. The serum iron increase has been studied in detail in swine.<sup>46</sup> The increase begins at approximately the 4th week of the deficiency and reaches its maximum (350 to 600  $\gamma$  per cent) between the 5th and 10th weeks. Following this the serum iron level tends to decline somewhat. Combined iron and pyridoxine deficiency results in an abnormally low serum iron level and in this combined deficiency hemosiderosis of the tissues is prevented. It would seem, then, that the ferremia of pyridoxine deficiency is caused by continued retention of iron at a time when its utilization for hemoglobin synthesis is at a minimum. The possibility exists that the absorption of iron is increased in pyridoxine deficiency.

Studies on the nature of the iron in pyridoxine deficiency reveal that approximately 95 per cent of the increased iron in the serum is in a nondialyzable, ferric state.<sup>46</sup>

Studies on the whole blood copper levels in dogs anemic because of pyridoxine deficiency reveal that this element is present in the low normal range and that

there is a slight rise after treatment with pyridoxine.<sup>41</sup> This would seem to indicate that copper, unlike iron, is not mobilized in the blood of the severely anemic animal.

Since it is possible that pyridoxine is essential for the synthesis of hemoglobin an iron-porphyrin complex, Lepkovsky and Parsons<sup>50</sup> studied the effect of pyridoxine deficiency on the synthesis of another iron-porphyrin complex, namely catalase, and found that there was no significant change in the catalase activity of the liver, kidney, and heart tissues of the rat. Thus there is no evidence to date that pyridoxine is directly concerned with the synthesis or metabolism of the porphyrins.

It has been shown that a relationship exists between pyridoxine and tryptophan metabolism. Pyridoxine-deficient rats,<sup>51, 52</sup> mice,<sup>52, 53</sup> dogs,<sup>48, 54</sup> and swine<sup>51, 52</sup> excrete in the urine abnormally large quantities of xanthurenic acid, a metabolite of tryptophan metabolism. The amount excreted is related to the amount of l-tryptophan ingested.<sup>52, 53, 55, 56</sup> It seems that as the protein intake is increased, the symptoms of the deficiency become more severe and the survival time is diminished.<sup>53, 54, 57</sup> Since a deficiency of either pyridoxine or tryptophan gives rise to anemia, the question arises as to whether the two substances combine to form a third which is essential to blood formation. If this were true then a deficiency of either one should result in the same morphologic changes in the blood. This cannot be the case since the anemia of tryptophan deficiency is normocytic, it is accompanied by a terminal leukopenia, the serum iron level is normal, hypoproteinemia exists, the bone marrow is normo- or hypoplastic and there is no hemosiderosis.<sup>57</sup>

In swine, combined pyridoxine and iron deficiency results in a greater anemia than does either deficiency alone.<sup>46</sup> The combined deficiency also results in a lower mean corpuscular volume. In one animal with the combined deficiency the hemoglobin reached 2 Gm. per cent and the mean corpuscular volume diminished to 24 cu.  $\mu$  (normal 58).

The anemia of pyridoxine deficiency responds to treatment with synthetic pyridoxine hydrochloride.<sup>38, 39, 41, 42, 44</sup> A reticulocytosis develops reaching a peak in two to six days and the reticulocytes may be increased to as much as 30 per cent. Following the reticulocytosis there is rapid restoration of the hemoglobin volume of packed red cells, and mean corpuscular volume toward normal. The magnitude of the changes in the blood following pyridoxine therapy appears to be related to the size of the dose given, the degree of anemia, and the route of administration. The highest reticulocyte increases and degrees of increase in hemoglobin and volume of packed red blood cells in pyridoxine-deficient swine were observed when the anemia was most severe and large amounts of pyridoxine were given intravenously. Doses as small as 10 and 20  $\mu$ g. per kilogram of body weight by mouth daily are ineffective in swine. A dose of 80  $\mu$ g. per kilogram of body weight by mouth daily is followed by a definite and pronounced response.

Many investigators have reported that although the anemia responds to pyridoxine, the addition of this factor alone to a synthetic diet is not sufficient to maintain the hemoglobin at optimal levels.<sup>38, 41, 44, 58</sup> There is at least one factor

in addition to pyridoxine which is found in liver and brewer's yeast and which is necessary to maintain hemoglobin production. The nature of this factor or factors is not known. It is not nicotinic acid, thiamine, riboflavin, pantothenic acid, choline, or inositol.

It has been demonstrated that the anemia does not respond to iron administration, either oral or intravenous, or to copper, cobalt, chlorophyll, chlorophyllin, concentrated liver extract, tryptophan, corn oil, hemoglobin, or hemin.<sup>46</sup>

Two derivatives of pyridoxine, pyridoxal, the 4-aldehyde of pyridoxine, and pyridoxamine, the 4-amine of pyridoxine, have been shown to be 5500 and 8000 times as active, respectively, in promoting the growth of *Streptococcus lactis* R as pyridoxine.<sup>59-61</sup> The relative value of these derivatives in the prevention and treatment of the anemia has not been studied.

It has been shown that pyridoxine, pyridoxal, and pyridoxamine function as the coenzyme of tyrosine, lysine, arginine, and glutamic acid decarboxylase.<sup>62-67</sup> The synthetic codecarboxylase prepared from pyridoxal has been found to be active with 3 of these enzymes. From these data it has been suggested that one of the functions of the vitamin B<sub>6</sub> group is to function as a coenzyme of amino acid decarboxylases. The relationship between this function and erythropoiesis is not obvious. It has been both asserted<sup>59, 65</sup> and questioned<sup>69</sup> that pyridoxine plays a role in the biological transamination reactions. Recently it has been rather conclusively shown that pyridoxal phosphate functions as the coenzyme of the glutamate-aspartate transaminase.<sup>70</sup> The function of the vitamin B<sub>6</sub> group in protein metabolism is therefore at least partially explained by its action in amino acid decarboxylation and transamination.

Pyridoxine has never been conclusively demonstrated to be essential to human nutrition. Pyridoxine deficiency anemia resembles pernicious anemia in several respects. In both conditions there is an increase in serum iron, hemosiderosis of the tissues, hyperplastic bone marrow, and neurological lesions. That they are not the same is evidenced by a microcytosis in one and a macrocytosis in the other. Pyridoxine anemia does not respond to liver extract<sup>46</sup> and it has been shown that this vitamin is not the extrinsic factor.<sup>29</sup> Pyridoxine anemia possesses some of the features of Mediterranean anemia. In both the red corpuscles are microcytic and hypochromic, serum iron is elevated, and iron-containing pigment is found in the tissues. Both types of anemia fail to respond to iron therapy. Goldman and Malavazos<sup>71</sup> have reported that pyridoxine is of value in the treatment of this anemia when given in conjunction with pregnancy-urine hormone. The present author studied the effect of pyridoxine alone on the same patients reported by Goldman and Malavazos and was unable to demonstrate an effect on either the serum iron or the anemia.<sup>72</sup>

Kark, Lozner, and Meiklejohn<sup>73</sup> treated 4 cases of pellagra, 1 case of "idiopathic" hypochromic anemia, and 1 case of nutritional macrocytic anemia with pyridoxine, and in none of their cases did the anemia respond to pyridoxine therapy. Moore, Minnich, Vilter, and Spies<sup>16</sup> studied 32 patients with hypochromic microcytic anemia associated with nutritional deficiency of various types and found that iron therapy alone produced a satisfactory reticulocyte response

and rate of hemoglobin generation. Brewer's yeast had no demonstrable effect in increasing the efficacy of iron therapy. They concluded that if pyridoxine deficiency was present in these patients it was not accompanied by hypochromic anemia with high serum iron levels. It has also been our experience that the serum iron level is low rather than high in deficiency states. However, if an associated iron deficiency is present this would be expected since a combination of the two deficiencies in the experimental animal results in a low rather than a high serum iron level.

In our experience pyridoxine has been found to be ineffective in the treatment of anemia of nephritis, aplastic anemia from various causes, the anemia of infections, pernicious anemia, Mediterranean anemia, and the anemia of malnutrition.<sup>72</sup>

Cantor and Scott<sup>74</sup> have reported 3 cases of agranulocytic angina of toxic origin which responded to the intravenous administration of pyridoxine hydrochloride. They suggest that pyridoxine acts by direct stimulation of the myelocytic elements of the bone marrow. Fishberg and Vorzimer<sup>75</sup> have reported that pyridoxine brings about a rapid and significant rise in the number of circulating granulocytes in human beings after a depression caused by thiouracil. These results are rather surprising since the white cells seem to be unaffected in experimental pyridoxine deficiency. Since granulocytosis may disappear spontaneously, especially when the offending agent is removed, acceptance of these claims concerning the value of pyridoxine in agranulocytosis must await further evidence.

**D. *Lactobacillus Casei* Factors.**—The various new factors described as having growth activity for *Lactobacillus casei* and related organisms are many. All of these factors have been found to have hemopoietic activity for one or more species of animals under certain conditions. Recently considerable clinical interest has developed in this group of substances because of the demonstration of the effectiveness of the synthetic *L. casei* factor in various macrocytic anemias in relapse. The early research in this field illustrates magnificently the importance to clinical medicine of so-called "ultrascientific" research. A recent review by Berry and Spies<sup>634</sup> entitled "The Present Status of Folic Acid" is available.

The literature is confusing and difficult to evaluate. Many divergent approaches have been used. These approaches have now, in part at least, converged. As further knowledge is gained and made known it can be expected that further simplification will take place.

In this review the various factors will be discussed separately although many of them have been shown to be identical (table 2). This is now a somewhat historical approach and is used with that purpose in mind as well as for clarity. The hematologic manifestations of these factors, or factor, will be discussed separately and by species.

1. **Norite eluate factor.**—In 1940 Snell and Peterson<sup>76</sup> reported that *Lactobacillus casei*, for maximal growth, required in addition to the basal media a norite filtrate factor and a norite eluate factor, both obtained from yeast. They demonstrated that the activity of the filtrate factor was due to the content of pyridoxine and biotin. Studies on the norite eluate factor were reported. "Solubilized" liver was found to be a rich source. The substance was heat stable, very labile to acids and

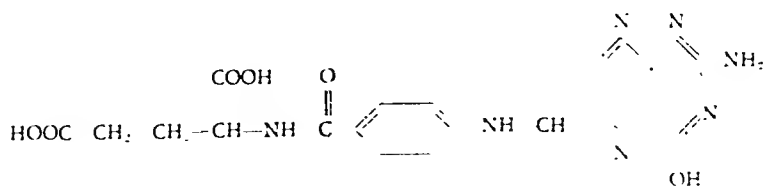
reducing agents, somewhat more stable to alkali and oxidizing agents, and resistant to enzyme action. The active substance was precipitated by phosphotungstic acid, basic lead acetate, copper sulfate, and sodium bisulfide. It was easily absorbed by lead sulfide and fuller's earth and was insoluble in all common organic solvents except glacial acetic acid, formamide, and dioxane. Hutchings, Bohonos, and Peterson<sup>77</sup> later prepared a purified concentrate of the eluate factor and presented evidence that the active principle was an acid and contained an amino group. They also found that their factor stimulated the growth of *Streptococcus lactis* R as well as *L. casei*. This factor is now considered to be identical with the one to be next described.

2. *Lactobacillus casei* factor from liver.—Stokstad<sup>78</sup> prepared a concentrate of a factor in liver which had microbiological activity for *L. casei*. He reported that the substance contained nitrogen, phosphorus, a pentose and guanine and concluded

TABLE 2.—*The Lactobacillus casei* Factors as Reported in the Literature

Factor	Authors	Source	Biological activity	Activity due to
Norite eluate factor	Snell, Peterson	Liver, Yeast	L. casei = S. lactis R	Pteroylglutamic acid
L. casei factor (liver)	Stokstad	Liver	L. casei = S. lactis R	Pteroylglutamic acid
Folic acid	Mitchell, Snell, Williams	Spinach	L. casei = S. lactis R	? Pteroylglutamic acid
S. lactis R factor	Kefauz, et al.	Liver	S. lactis R > L. casei	? Pterioic acid
Yeast factor	Stokstad	Yeast	L. casei > S. lactis R	"
Fermentation factor	Hutchings, et al.	Unstated	L. casei > S. lactis R	Pteroyltriglutamic acid
Vitamin M	Day, Langston, Shukers	Liver, Yeast	Monkey	Pteroylglutamic acid
Xanthopterin	Simmons, Norris	Synthetic	Salmon	Related chemically to pteroylglutamic acid
Vitamin B <sub>12</sub>	Hogan, Parrott	Liver	Chick	Pteronic acid
Vitamin B <sub>12</sub> Conjugate	Binkley, et al.	Yeast	Chick	Pteroylglutamic acid
Vitamin B <sub>12</sub> Conjugase	Mims, et al.	Liver		Structure unknown
Vitamin B <sub>12</sub>	Briggs, et al.	Liver	Chick	Pteroylglutamic acid
Vitamin B <sub>12</sub>	Briggs, et al.	Liver	Chick	Pteroylglutamic acid
Pyracin	Scott, et al.	Synthetic	None	
Thymine	Spies, et al.	Synthetic	Man, S. lactis R	?

that the substance was a nucleotide. He found that the factor was partially replaceable by thymine and guanine. Later work<sup>79</sup> with purer concentrates showed it to be free of phosphorus. This compound has now been isolated and synthesized.<sup>80, 81</sup> Both the natural and synthetic compounds are equally active when assayed by *L. casei* or *S. lactis* R. The formula has recently been announced<sup>655</sup> and is as follows:



N-4-[1,2-amino-3-hydroxy-6-pyridyl] methyl amino benzyl glutamate-202

Pteroylglutamic acid is the chemical name which has been suggested for this compound. The synthetic substance has been placed on the market under the name "folic acid."

3. *Folic acid*.—Mitchell, Snell, and Williams<sup>82</sup> prepared a concentrate from spinach which stimulated the growth of *Streptococcus lactis* R. This compound was 100 times more active than Stokstad's original preparation. From diffusion experiments the compound appeared to have a molecular weight of about 500. Later the molecular weight was reported to be more nearly 400.<sup>83</sup> Their preparation was essentially a pure compound and because it was obtained from a leafy material was named folic acid. The active material has now been obtained from liver, yeast, milk, casein, peptone, and other natural sources. Williams and his group<sup>82-85</sup> have obtained highly purified concentrates which are 137,000 times more active than a standard liver extract. They have reported in detail their concentration methods as well as absorption studies and chemical and physiological properties of the active material. The compound is markedly unstable to oxidation, reduction, acid, alkali, light, dry heat, acetylation, esterification, methylation, benzoylation, nitrous acid, bromine, and hypobromite. It is highly soluble in water, glacial acetic acid, and liquid ammonia but is essentially insoluble in methanol, ethanol, butanol, acetone, ether, dioxine, benzene, petroleum ether, and chloroform. The ammonium salt is quite soluble in aqueous alcohol and is very soluble in water. They have indicated that the formula may be approximately  $C_{15}H_{15}O_8N_5$ . Absorption studies seem to indicate that folic acid may be converted by various treatments to substances which are very closely related chemically but which have greatly reduced physiological properties. Minor changes in structure seem to produce great changes in the activity for various organisms. This fact may be of significance in considering the next three factors to be described.

Thymine replaces folic acid if given in sufficient quantities.<sup>78, 87, 88</sup> Approximately 5,000 times more thymine is required than folic acid and although thymine can completely replace folic acid in the nutrition of *S. lactis* R it does not completely replace folic acid activity in the nutrition of *L. casei*. Stokes<sup>87</sup> has suggested that folic acid participates directly or indirectly as a coenzyme in the synthesis of thymine or a related compound in the lactic acid streptococci. Folic acid has not been detected in streptococci grown in thymine medium. Hypoxanthine, alloxazine, alloxantine, guanidine, theobromine, xanthopterin, alloxan, allantoin, and uric acid have slight activity at high concentrations. Absorption studies indicate that folic acid contains in its structure a unit very similar to xanthopterin.

Originally folic acid was considered to be a different substance from the norite eluate factor and the liver *Lactobacillus casei* factor of Stokstad because the compound contained no phosphorus and was active for *Streptococcus lactis* R. These two differences were later reconciled when Stokstad<sup>79</sup> and Hutchings et al.<sup>77</sup> demonstrated that their factors did not contain phosphorus and when it was demonstrated that the norite eluate factor stimulated the growth of *Streptococcus lactis* R<sup>77</sup> and that folic acid was equally effective for *L. casei*.<sup>82, 89, 90</sup> However, since the folic acid preparation was not entirely pure it is possible that it contained several of the factors to be mentioned in addition to folic acid. Hutchings, Stokstad,

Bohonos, and Slobodkin<sup>80</sup> have presented evidence on the basis of absorption spectra that folic acid is different from the liver compound which they isolated.

4. *Streptococcus lactis* R factor of Keřesztesy, Rickes, and Stokes.—These workers<sup>90</sup> isolated a growth factor "from various types of extracts and liver preparations" which they believed was neither folic acid nor the norite eluate factor. The substance was found to be 2500 times more active for *Streptococcus lactis* R than for *Lactobacillus casei*. Sebrell<sup>159</sup> noted that this factor was inactive in alleviating the anemia and leukopenia occurring in rats following the administration of sulfaguanidine in addition to a purified diet.

Using the same methods of synthesis as for pteroylglutamic acid except for the substitution of p-aminobenzoic acid for p-aminobenzoyl-l (+)-glutamic acid, a compound, pterioic acid, has been obtained which is active for *Streptococcus lactis* R but inactive for *Lactobacillus casei* and the chick.<sup>635</sup> It is possible that this factor is identical with Keřesztesy's factor.

5. *Yeast factor of Stokstad*.—Stokstad<sup>79</sup> isolated a compound from yeast which on the basis of microbiological assay he believed to be distinct from the liver *L. casei* factor. The preparation from yeast was found to be only half as active for *Streptococcus lactis* R as for *L. casei*.

6. *Factor of Hutchings et al.*—Hutchings, Stokstad, Bohonos, and Slobodkin<sup>80</sup> isolated a factor in crystalline form from an unstated source which was active for *L. casei* and *S. lactis* R and also active in the nutrition of the chick but which they believed on the basis of absorption spectra to be different from folic acid and the *L. casei* factor isolated from liver. This new compound was 85 to 90 per cent as active as that from liver when assayed with *L. casei*, but only 6 per cent as active as the liver compound by *S. lactis* R assay. This substance is known as the "fermentation compound" and has been found to differ from pteroylglutamic acid in that it contains two additional glutamic acid groups.<sup>636</sup> Day et al.<sup>91</sup> found that treatment of this preparation with the enzyme solution of Mims et al.<sup>110</sup> greatly increased its activity toward *S. lactis* R.

7. *Vitamin M*.—In 1935 Day, Langston, and Shukers<sup>92</sup> reported that young monkeys maintained on a diet of casein, whole wheat, polished rice, cod liver oil, orange, and a salt mixture supplement lost weight, developed diarrhea and gingivitis, and died between the 26th and 100th day of a fulminating fatal blood disorder characterized by anemia, leukopenia, and thrombocytopenia. Likewise, monkeys maintained on a modified Goldberger black-tongue-producing diet consisting of corn, cowpeas, casein, cottonseed oil, cod liver oil, and salt mixture developed a similar syndrome.<sup>93</sup> These observations have been amply confirmed by other investigators.<sup>94-105</sup> The addition to the diet of ascorbic acid, nicotinic acid, riboflavin, thiamine, copper, iron, various types of concentrated liver extract including "anahaemin," pantothenic acid, pyridoxine, choline, pimetic acid, glutamine, inositol, p-aminobenzoic acid, banana, and heated liver extract failed to prevent the nutritional cytopenia.<sup>93 102 106 107</sup> The deficient diet supplemented with either dried brewer's yeast or two grams of liver extract (Cohn fraction G), daily, supported good growth, promoted normal body development, and maintained a normal blood picture over long periods of time.<sup>108</sup> When liver

extract was added to the diet of a deficient animal with profound anemia and leukopenia a dramatic reticulocytosis occurred and was followed by ultimate recovery. The authors proposed the term "vitamin M" for the factor which prevented the nutritional cytopenia in the monkey.<sup>106</sup> Wilson, Doan, Saslaw, and Schwab<sup>102</sup> reported that the addition of folic acid concentrate to the diet restored normal white cell equilibrium. Totter and co-workers<sup>108</sup> assayed a number of substances for folic acid by the *S. lactis* R method and were unable to find a correlation between folic acid and vitamin M content. However, materials which contained very little folic acid but which were good sources of vitamin M were found to give an increase in *S. lactis* R assay when incubated with an enzyme preparation from rat liver.<sup>109-112</sup> Following such an enzymatic conversion of the potential *S. lactis* R-stimulating factor there was excellent correlation with the vitamin M activity. They then suggested that vitamin M, vitamin B<sub>6</sub> conjugate, the potential *S. lactis* R-stimulating factor and the factor antagonistic to the succinylsulfathiazole effect in rats were probably similar if not identical.<sup>113</sup> They have now demonstrated that vitamin M-deficient monkeys respond to the crystalline *L. casei* factor of Hutchings et al.<sup>91, 114, 637</sup>

8. *Xanthopterin*.—Xanthopterin, the yellow pterin pigment of butterfly wings, was isolated from the wings of the *Colias philodice* in 1936 by Schöpf and Becker.<sup>115</sup> The compound was synthesized by Purmann in 1940<sup>116, 117</sup> and recently a convenient method for its synthesis has been reported by Totter.<sup>118</sup> The compound has been isolated from human urine and liver.<sup>119, 120</sup> Urinary xanthopterin is known as uropterin.

Sir Frederick Gowland Hopkins first suggested that pterins play a role in hemopoiesis.<sup>121</sup> In 1936 Tschesche and Wolf<sup>122</sup> reported that pterins and particularly xanthopterin have hemopoietic activity when administered to rats made anemic on a diet of goat's milk. Simmons and Norris<sup>123</sup> reported in 1941 that the anemia occurring in Chinook salmon maintained on a high protein diet in which the vitamin B complex was supplied in the form of yeast responded to injections of 50  $\gamma$  of crystalline xanthopterin. This has since been confirmed.<sup>124</sup> The photoisomer of xanthopterin was found to be lethal in a similar dose.

Evidence that xanthopterin is closely related to folic acid has been supplied. Wright and Welch<sup>125</sup> in experiments with surviving rat livers have shown that, following the incubation of rat liver with xanthopterin, more folic acid is found on microbiological assay of the digestion mixture than is present in a similar amount of rat liver alone. They also found a substance in urine which was stable to both heat and acid and which occurred in both a free and combined form.<sup>126</sup> When the substance was incubated with fresh rat's liver there was a demonstrable increase in the folic acid content. The evidence suggested that this compound is related to xanthopterin. Williams and his group, from absorption studies of xanthopterin and folic acid, have suggested that folic acid contains a structural unit very similar to xanthopterin.<sup>83, 86, 128</sup> Bloom et al.<sup>127</sup> state that the ultraviolet absorption characteristics of vitamin B<sub>6</sub> coupled with the nitrogen content of the compound suggest the presence in the molecule of a pyrimidopyrazine ring structure such as a pterin. Totter and co-workers<sup>108, 129</sup> found that the addition of

2.5 to 10 mg. of synthetic xanthopterin to vitamin M-deficient monkeys resulted in a reticulocytosis in 3 to 6 days which lasted 2 to 5 days. White and red cell counts increased to normal in 3 to 13 days and remained normal for varying periods. Synthetic xanthopterin alone when given to prevent cytopenia failed to protect completely but did delay the onset of nutritional cytopenia. In one animal given heated liver (previously shown to be ineffective by itself) plus xanthopterin, the white and red cell counts were still normal after 71 days. Cessation of xanthopterin therapy resulted in a prompt return of the cytopenia and resumption of this therapy resulted in a response similar to the first. They also noted that the livers obtained from vitamin M-deficient monkeys were low in preformed folic acid and that when the livers were incubated with xanthopterin and yeast or yeast alone there was a rise in the folic acid content.<sup>169</sup> These authors concluded that the monkey requires xanthopterin as well as unidentified factors in liver for hemopoiesis.

Totter and Day<sup>130</sup> reported that in rats xanthopterin was effective in alleviating the leukopenia and counteracting the growth inhibition produced by adding 1 per cent succinylsulfathiazole to a synthetic diet complete in all known members of the vitamin B complex. Others have been unable to confirm this<sup>125, 131, 132</sup> and the authors now state that they are unable to repeat their own work. O'Dell and Hogan<sup>133</sup> have stated that xanthopterin is inactive when fed to nutritionally anemic chicks, and Totter, Mims, and Day have found that chicken liver fails to produce any extra folic acid from xanthopterin alone.<sup>169</sup>

9. *Vitamin B<sub>c</sub>*.—In 1940 Hogan and Parrott<sup>131</sup> presented evidence for the existence of an unidentified factor necessary in addition to the known vitamins for the prevention of anemia in chicks. A factor in liver designated vitamin B<sub>c</sub> was found to prevent the development of the anemia. This anemia has now been characterized and studied, and the preventive factor isolated in crystalline form.<sup>135</sup> All the available evidence indicates that the substance is identical with the *L. casei* factor isolated from liver and the norite eluate factor and is at least similar to folic acid.

O'Dell and Hogan<sup>133</sup> in 1943 pointed out that vitamin B<sub>c</sub> was acidic in nature, formed salts with heavy metals, was destroyed by mineral acids, stable to alkali, adsorbed on fuller's earth and norite, eluted by ammonia, destroyed by oxidation, and insoluble in the common organic solvents. Analysis of an ash-free specimen gave the following percentage composition: C 52.44, 52.46; H 4.28, 4.49; N 19.8, 19.6. The ultraviolet absorption characteristics coupled with the nitrogen content suggest the presence in the molecule of a pyrimidopyrazine ring structure such as a pterin.<sup>127</sup> A comparison of the ultraviolet absorption curves for xanthopterin and vitamin B<sub>c</sub> brings out very striking dissimilarities although in general they are quite similar. The fact that synthetic *L. casei* factor is effective in preventing the anemia in chicks,<sup>91</sup> that crystalline vitamin B<sub>c</sub> is a highly active growth factor for *L. casei*<sup>135</sup> and is effective in correcting the anemia in rats maintained on a purified diet supplemented with sulfone compounds<sup>136</sup> together with the demonstration that the vitamin B<sub>c</sub> potency of the livers from rats receiving succinylsulfathiazole is markedly reduced<sup>137</sup> is good evidence that the *L. casei* liver factor and vitamin B<sub>c</sub> are identical. Furthermore, both factors have similar solubilities and both are adsorbed on fuller's earth at acid pH levels.

10. *Vitamin B<sub>6</sub> conjugate*.—Binkley et al.<sup>138</sup> observed that certain yeast extracts were highly active in vitamin B<sub>6</sub> activity as measured in the anemic chick but they had little potency in stimulating the growth of *L. casei*. Only about 2 to 5 per cent of the chick antianemic activity could be accounted for in terms of microbiological growth effect on either *L. casei* or *S. lactis* R. The methods used for isolating vitamin B<sub>6</sub> failed when applied to yeast. They noted, however, that the concentrates of the chick antianemic factor from yeast which were essentially inert in stimulating growth of *L. casei* became highly active in microbiological growth effect following enzymatic digestion. Procedures used for the isolation of vitamin B<sub>6</sub> when applied to such digests yielded a pure crystalline compound which had the same growth-stimulating activity on *L. casei* and *S. lactis* R as vitamin B<sub>6</sub> from liver and also produced a comparable effect on the blood picture and growth of chicks. The products from the two sources also had the same color, crystalline appearance, solubilities, charring points, crystallographic pattern, ultraviolet absorption spectra, and elementary chemical analysis. They referred to the chick antianemic factor in yeast as vitamin B<sub>6</sub> conjugate and the enzyme which formed vitamin B<sub>6</sub> from it, vitamin B<sub>6</sub> conjugase.<sup>139, 141</sup> Subsequent work resulted in the isolation of vitamin B<sub>6</sub> conjugate.<sup>142</sup> The pure crystalline substance has an elementary composition of C 49.61 per cent; H 5.36 per cent; N 14.79 per cent. Comparison of the specific ultraviolet absorption properties of this compound with vitamin B<sub>6</sub> reveals that both have the same chromophoric groups. It appears that the molecular size of vitamin B<sub>6</sub> conjugate is 2.8 times that of vitamin B<sub>6</sub>. The data demonstrate that the vitamin B<sub>6</sub> present in the conjugate molecule as calculated from the ultraviolet absorption data and as found by enzymatic microbiological assay is available to the chick. The new crystalline product is only slightly stimulating to the growth of *L. casei* and *S. lactis* R and can be clearly differentiated from the various *L. casei* factors on this basis as well as by the ultraviolet absorption constants. This substance is now known to be pteroyl-heptaglutamic acid.<sup>636</sup>

Vitamin B<sub>6</sub> conjugase has been found to be widely distributed in nature.<sup>140, 141, 638, 639</sup> Hog kidney, liver, small intestine, and beef liver are rich sources. It occurs in sweet almond and to a lesser extent in potatoes. Only traces have been found in molds and none in yeast.

11. *Vitamins B<sub>10</sub> and B<sub>11</sub>*.—Briggs et al.<sup>143-145</sup> have demonstrated the existence of two water-soluble vitamins which they believe to be separate from the various factors just described and which are needed by the chick for maintenance of normal hemoglobin values, proper feather formation, and normal growth. The factor essential for feather formation has been named B<sub>10</sub> and the factor necessary for growth B<sub>11</sub>. Both factors are effective in preventing the development of anemia. The anemia is stated to be macrocytic and accompanied by leukopenia. From their studies they conclude that both B<sub>10</sub> and B<sub>11</sub>, "although distinct entities, seem to be related chemically to the various factors with vitamin B<sub>6</sub> activity, since the properties are so similar and because compounds with vitamin B<sub>6</sub> activity have some vitamin B<sub>10</sub> and B<sub>11</sub> activity when fed alone to the chick."

It has now been demonstrated that the addition of 25  $\gamma$  of synthetic folic acid

(pteroylglutamic acid) per 100 grams of basal ration entirely prevents the reduced growth, poor feathering condition, and low hemoglobin and hematocrit values consistently obtained when the basal ration is fed to chicks.<sup>610</sup> Thus all vitamin B<sub>10</sub> and B<sub>11</sub> activity is due to folic acid.

Briggs et al.<sup>145</sup> have suggested the existence of another unknown factor necessary to maintain normal hemoglobin formation in the chick in addition to those described above.

12. *Pyracin*.—Scott et al.<sup>146, 147</sup> have presented evidence that either the lactone of 2-methyl-3 hydroxy-4 carboxy-5 hydroxymethylpyridine ( $\gamma$ -pyracin) is required in addition to the *Lactobacillus casei* factor for the complete prevention of the macrocytic anemia that develops in chicks fed a purified diet. The results of hematological studies showed that when the *L. casei* factor alone was added to the diet, normocytic, hypochromic anemia developed. When  $\beta$ -pyracin was added alone, a macrocytic, normochromic anemia developed. Furthermore, they demonstrated that when pure *L. casei* factor and pyracin were incubated with liver there was a marked increase in the *S. lactis* R-stimulating potency.<sup>148</sup> They have suggested that pyracin may form a conjugate with the *L. casei* factor or that it may enter into an enzyme system which brings about the conversion. These results have not been confirmed by others<sup>17</sup> and Hutchings, Oleson, and Stokstad<sup>611</sup> have found that the addition of  $\beta$ -pyracin is not necessary for growth or hemoglobin formation in addition to the synthetic liver *Lactobacillus casei* factor.

13. *Hematological manifestations of the deficiency*.—*a. Rat*.—Rats fed sulfonamides at a level of 1 to 2 per cent in purified diets develop a severe granulocytopenia, leukopenia, thrombocytopenia, and normocytic anemia in 4 to 6 weeks.<sup>151, 152–154</sup> The white cell count falls from a level of 10 to 16 thousand down to 1 to 4 thousand. Blood smears are reported as showing marked abnormalities in the size, shape, and staining reactions of the red cells and an increase in the number of nucleated forms occurs.<sup>150</sup> The anemia has been reported by some authors as being hypochromic.<sup>156</sup> The granulocytopenia is pronounced and there may also be a reduction in the absolute number of lymphocytes. Severe anemia may be produced regularly in rats fed a sulfasuxidine-containing purified diet and subjected to hemorrhages, whereas rats fed a purified diet alone do not develop anemia when bled to the same extent.<sup>155</sup> Granulocytopenia has been observed in a small percentage of rats fed purified diets without sulfonamides.<sup>156</sup>

The histologic picture of the bone marrow varies from almost complete aplasia to intense hyperplasia.<sup>157</sup> The total nucleated cell count is only slightly less than normal. However, the majority of marrow cells are undifferentiated primitive forms with relative increases in myeloblasts and early erythroblasts. Myelocytes, adult granulocytes, late erythroblasts and megakaryocytes practically disappear. Thus there is a maturation arrest in the early stages of the development of the three cellular elements of the blood.<sup>158, 159</sup>

Crystalline *L. casei* factor has been found to have a preventive as well as a corrective action on the anemia, leukopenia, and thrombocytopenia.<sup>152</sup> A concentrate of vitamin B<sub>6</sub> has also been shown to be effective.<sup>156</sup>

Rats fed thiouracil in a purified diet develop anemia and, in lesser incidence,

leukopenia. Animals which receive, concomitantly, thyroxin injections or thyroid powder become granulocytopenic and leukopenic. The granulocytopenia and leukopenia of these rats may be corrected by treatment with synthetic *L. casei* factor.<sup>642</sup>

*b. Chick.*—The characteristics of the anemia, the leukopenia, and the thrombocytopenia developing in chicks on purified rations deficient in vitamin B<sub>12</sub> are summarized in table 3. All workers are in agreement that the anemia is macrocytic<sup>134, 147, 160-162</sup> and that the mean corpuscular hemoglobin is increased. There is no agreement as to the mean corpuscular hemoglobin concentration. Various workers have found that the anemia is hypochromic,<sup>160, 162</sup> normochromic,<sup>147</sup> or hyperchromic.<sup>134, 162</sup>

Anemia is detectable after 7 days. Thereafter it develops rapidly and after 28 days on the deficient ration the anemia is pronounced. After about 9 days the

TABLE 3.—*The Cytopoietic Effect of Crystalline Vitamin B<sub>12</sub> on Chick Blood*

	Normal	B <sub>12</sub> Def.	B <sub>12</sub> Added
R.B.C. millions cu. mm. . . . .	2.27	0.93	2.11
Hemoglobin Gm. % . . . . .	7.74	4.76	8.03
Volume packed R.B.C. cc./100 cc. . . . .	31.2	15.0	30.7
Mean Corpuscular Volume cu. u. . . . .	137	161	143
Mean Corpuscular Hemoglobin. . . . .	34	51	38
Mean Corpuscular Hemoglobin conc. % . . . . .	25	32	26
W.B.C. per cu. mm. . . . .	29,935	7,690	24,440
Neutrophils . . . . .	5,733	5,395	5,965
Eosinophils . . . . .	449	274	244
Basophils. . . . .	659	197	415
Lymphocytes . . . . .	22,406	1,756	16,961
Monocytes . . . . .	688	68	855
Thrombocytes cu. mm. . . . .	31,180	18,020	32,620

immature red cells are increased and polychromatophilia and basophilia are evident. Macrocytes with a great variety of sizes and shapes appear on about the 14th day. By the 21st day numerous normoblasts, pronormoblasts, and myeloblasts are evident. Active mitotic figures are common. At 28 days the nucleated red blood cells present a moderate to marked anisocytosis and poikilocytosis. Polychromasia, basophilia, and macrocytosis are extremely marked. In addition the nuclei of many of the immature cells become eccentrically placed and the nuclear chromatin breaks into fragments within the cells.

The pattern of the leukocytes deviates considerably from the normal. After about 9 days the distribution, size, number, and staining reactions of all white cells become progressively variable as leukopenia develops. The neutrophils appear pyknotic, the nuclei become hypersegmented and acquire a lighter staining characteristic. Vacuoles appear in the cytoplasm of the lymphocytes after the 14th day. By the 28th day the leukopenia is pronounced. The absolute number of neutrophils is maintained. Lymphopenia is extreme and there is also a reduction in the number of eosinophils, basophils, and monocytes.

The thrombocytes undergo considerable alteration in size, shape, and numbers. Karyorrhexis and pyknosis develop. After 3 to 4 weeks there is an increase in the vacuolation of the cytoplasm along with a general swelling of the granules. The degree of thrombocytopenia is extremely variable but generally is not extreme.

The addition of 5 to 10 micrograms of pure crystalline vitamin B<sub>12</sub> per 100 grams of basal ration prevents the appearance of severe anemia. The addition of 20 to 40 micrograms per 100 grams of basal ration gives nearly optimal protection against anemia and thrombocytopenia, whereas 20 to 200 micrograms is sufficient for the maintenance of normal hemopoiesis in 4 weeks old growing chicks.<sup>162</sup> Synthetic *L. casei* factor from liver has also been shown to be effective in preventing the anemia.<sup>81</sup>

c. *Monkey*.—Monkeys maintained on a diet of refined foodstuffs (vitamin M deficiency)<sup>92, 93</sup> or on a purified diet supplemented with nine crystalline members of the vitamin B complex plus ascorbic acid but deficient in the *L. casei* factor and related substances develop anemia, leukopenia, and thrombocytopenia.<sup>163, 164</sup> The anemia is reported to be normocytic.<sup>165</sup> The most characteristic feature of the syndrome is the leukopenia.<sup>165</sup> The total white count is usually reduced from a normal value of 15,000 to 2,000–3,000 per cu. mm. A few animals have developed white counts of less than 1,000 per cu. mm. All of the white blood cell types are involved although there is considerable variation from animal to animal in regard to cell type distribution. However, there is almost invariably an absolute neutropenia as well as an absolute lymphopenia.

The peripheral blood smears have not been studied carefully from the standpoint of red blood cell morphology. It has been stated<sup>161</sup> that the bone marrow of animals dying from severe leukopenia shows a relative and absolute hypoplasia of the myeloid elements but detailed studies of the bone marrow have not been reported.

d. *Human subjects*.—The value of folic acid (pteroylglutamic acid) in various types of macrocytic anemia in relapse has now been adequately demonstrated and repeatedly confirmed.

Spies et al.<sup>166</sup> observed a reticulocyte response and an increase in the red cell count in patients with macrocytic anemia in relapse. The drug was given orally to 4 patients in a dose of 100 to 150 mg. daily and parenterally to 5 patients in a dose of 20 to 50 mg. It was not stated whether these patients had nutritional macrocytic anemia or Addisonian pernicious anemia.

Moore, Bierbaum, Welch, and Wright<sup>168</sup> noted clinical and hematologic remissions in 2 patients with Addisonian pernicious anemia following the daily oral administration for 10 days of 30 mg. and 100 mg. of the synthetic material, respectively. One of the patients had an initial red count of 1.2 million cells per cu. mm. This patient developed a reticulocyte peak of 40 per cent on the 7th day of therapy. The other patient had an initial red blood cell count of only 0.7 to 0.95 million. In this patient a maximal reticulocytosis of 44.5 per cent was reached on the 8th day. Leukopenia and thrombocytopenia were also present and were corrected by the administration of *L. casei* factor. The response of this patient is shown in figure 1.

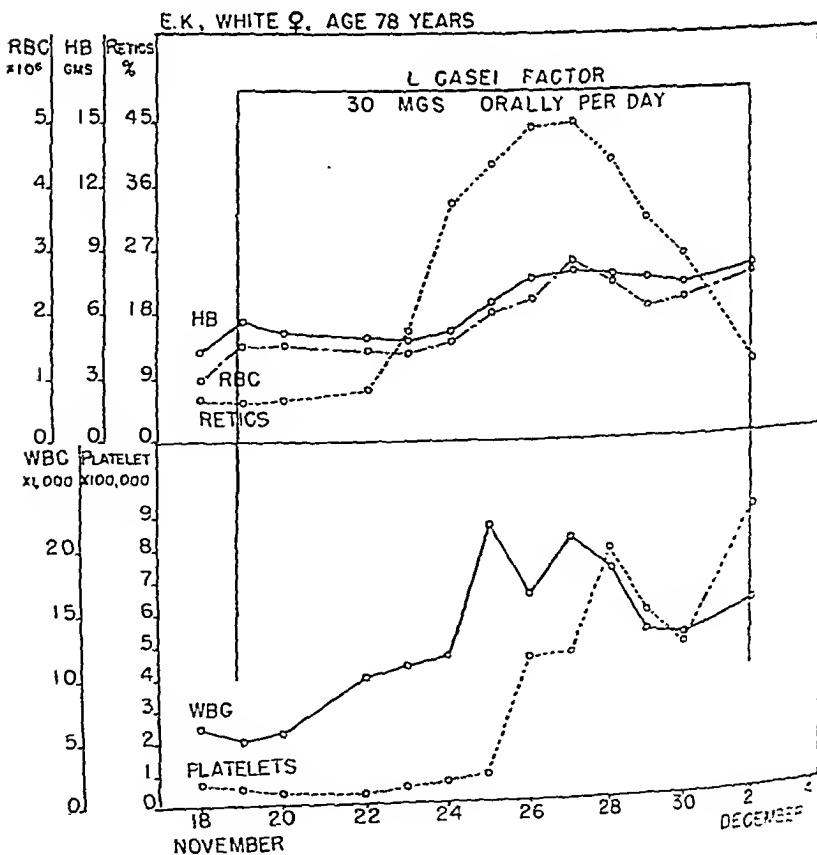
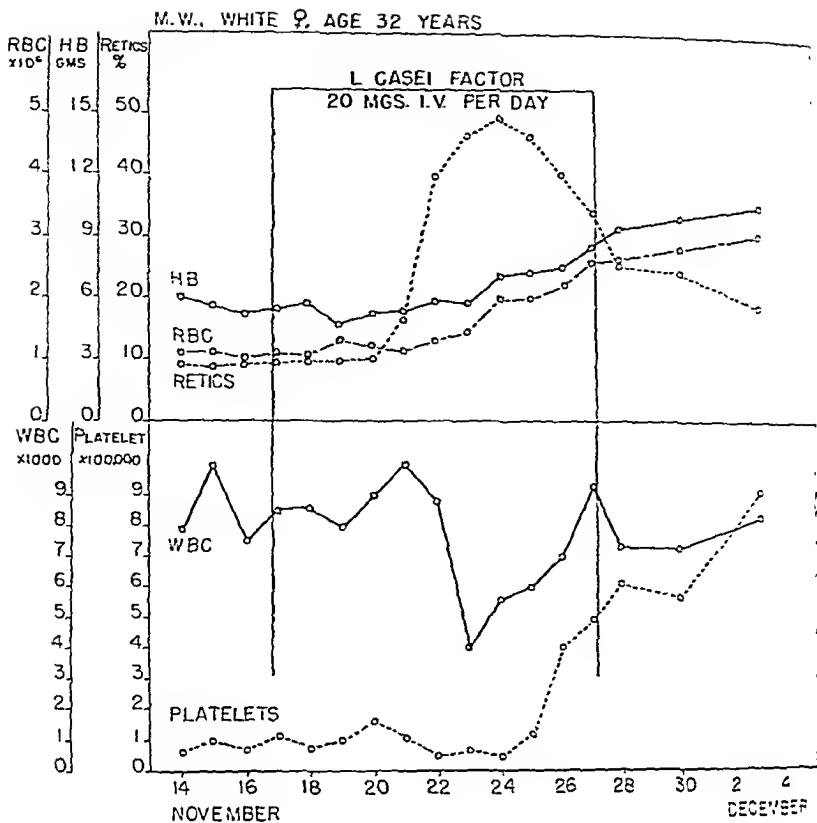


FIG. 1

Observations on the effectiveness of synthetic folic acid in the treatment of Addisonian pernicious anemia have now been confirmed many times.<sup>643-648</sup> The first change noted is an improvement in the general well-being of the patient on about the 3rd day of therapy. This is followed in a day or so by a marked reticulocytosis reaching a maximum between the 6th and 10th days of therapy. There is a gradual rise in leukocytes, platelets, red cells, hemoglobin, and volume of packed red cells to normal. The immediate response is quantitatively and qualitatively equal to that following liver extract therapy. The effect of synthetic folic acid on the neurological manifestations of pernicious anemia remains to be determined. The exact minimal and optimal doses of folic acid have not been determined but a daily dose of 5 to 10 mg. either orally or parenterally will often produce a maximal response.

Synthetic folic acid has been found to be effective in alleviating the hematologic as well as many of the other manifestations of sprue. This observation was first made by Darby and Jones<sup>167, 649</sup> and has since been confirmed by them as well as by others.<sup>168, 644, 619-652</sup> The substance is effective in both nontropical and tropical sprue. The administration of 15 to 200 mg. daily of the drug is followed by a reticulocytosis and rise in the leukocytes, platelets, red count, hemoglobin, and volume of packed red cells to normal. The glossitis disappears in 3 to 4 days, after which there is rapid regeneration of papillae. There is an improved sense of well-being, an improvement in appetite, and a decided gain in body weight. The sternal marrow returns to normal. Improvement in the absorption of glucose and water-soluble vitamins has been noted. Spies and his associates in Cuba and Puerto Rico have observed a profound effect on the alimentary tract of persons with sprue<sup>651</sup> and state: "Roentgenograms of the gastro-intestinal tract showed that the highly irritated bowel tends to become normal. There is a marked improvement in the diarrhea. The number of stools per day decreases and the feces, loose and bulky before therapy, usually become formed and essentially normal in appearance." Darby, Jones, and Johnson<sup>652</sup> failed to observe improvement in the absorption of fat in 2 cases during a 2 month observation period. Spies likewise has found that the steatorrhea of the more chronic cases is little affected.<sup>651</sup>

Synthetic folic acid has been found to be effective in the treatment of nontropical nutritional macrocytic anemia,<sup>613, 614</sup> macrocytic anemia of infancy with megaloblastic bone marrow,<sup>653-655</sup> macrocytic anemia of pregnancy,<sup>168, 611</sup> macrocytic anemia associated with alcoholic cirrhosis of the liver,<sup>644</sup> and macrocytic anemia associated with carcinoma of the stomach.<sup>611</sup>

Watson, Sebrell, McKelvey, and Daft<sup>171</sup> noted an increase in the leukocyte count after the daily oral administration of 5 mg. of *L. casei* factor to 7 patients who had developed leukopenia following roentgen-ray therapy. Berry, Spies, and Doan administered synthetic folic acid parenterally to leukopenic malnourished patients and noted a transient rise in the leukocytes in some cases.<sup>657</sup>

No response to folic acid has been observed in cases of aplastic anemia, leukemia, or iron deficiency anemia.<sup>171, 644, 656</sup> Folic acid has not been found to be of value in correcting the leukopenia of influenza, or the panhematopenia of myelophthisic and idiopathic states.<sup>654</sup> Newman and Jones<sup>658</sup> noted the failure of crystalline

folic acid to prevent the development of agranulocytosis in a patient receiving thiouracil.

Spies and co-workers<sup>659-661</sup> have administered large doses (4 to 12 grams) of thymine (5-methyl uracil) to 10 cases of Addisonian pernicious anemia. The clinical and hematological improvement was found to be similar to that which follows the administration of folic acid to such patients. The exact mode of action of thymine is obscure but the authors speculate that perhaps folic acid acts as an enzyme or coenzyme in the synthesis of thymine or a thymine-like compound.

The recent advances in the therapy of Addisonian pernicious anemia just described lead naturally to questions as to the identity of the extrinsic factor, the intrinsic factor, and the antipernicious anemia substance in liver as postulated by Castle. It can be stated that the folic acid is probably not the extrinsic factor alone since it acts orally in the absence of normal human gastric juice and is effective parenterally. However, as Moore<sup>168</sup> points out, caution must be used in making this differentiation since there is no information as to what effect Castle's extrinsic factor would have were it to be injected intravenously in pure form. That folic acid is identical with the effective substance in liver is unlikely for two reasons. At least 2 to 5 mg. daily are necessary to obtain an effective response. Good responses with as little as 0.7 mg. of highly purified liver extract given intravenously for 3 days have been obtained.<sup>169</sup> Secondly, purified commercial liver extract does not possess significant *L. casei* activity to explain its therapeutic activity.<sup>123, 170</sup> Welch, Heinle, Nelson, and Nelson<sup>662</sup> have recently made several interesting observations. They found that pteroylglutamic acid (folic acid) was released from pteroylheptaglutamate (vitamin B<sub>12</sub> conjugate) in a normal individual but not in a patient with pernicious anemia. When 2 pernicious anemia patients were given injections of purified liver extract the urinary excretion of pteroylglutamic acid (*L. casei* factor) doubled. The addition of purified liver extract to sternal marrow from patients both with and without pernicious anemia frequently augmented the formation of *L. casei* factor from added pteroylheptaglutamate (*in vitro* at pH 4.5 but not at pH 7.0). Surprisingly, however, when pteroylheptaglutamic acid was incubated with normal human gastric juice no free folic acid was released and when a patient with pernicious anemia in relapse was given the heptaglutamate orally for 11 days with normal human gastric juice no reticulocyte response occurred. This latter finding is difficult to explain but in general the results seem to indicate that in pernicious anemia it is the conjugase system which is at fault and that a constituent of liver extract may be a component of the conjugase system or may counteract the effect of conjugase-inhibiting substances. These findings would account, in part at least, for the failure of pernicious anemia patients to derive adequate amounts of folic acid from the diet since in nature the compound may occur principally in the conjugated form.

E. Extrinsic Factor.—It has now been 17 years since Castle first presented evidence concerning a dietary factor which when incubated with a factor in normal human gastric juice formed a substance effective in the treatment of pernicious anemia.<sup>172-175</sup> As each new B vitamin has appeared it has been tested in the hope that it might be the active factor. It has now been demonstrated that thiamine:

riboflavin,<sup>20, 176-178</sup> nicotinic acid, pyridoxine, pantothenic acid, p-aminobenzoic acid, choline, inositol, biotin, and xanthopterin are all inactive given either singly or in combination.<sup>20</sup> The possible identity or relationship of the extrinsic factor with one of the substances of the *L. casei* factor group, especially pteroylheptaglutamic acid, has just been discussed.

Beef muscle,<sup>172</sup> yeast,<sup>179</sup> wheat germ,<sup>180</sup> milk,<sup>181</sup> purified casein,<sup>20</sup> liver,<sup>182</sup> eggs,<sup>19</sup> and rice polishings<sup>19</sup> have been shown to contain significant amounts of the factor. Several commercial "vitamin-free" casein preparations contain extrinsic factor according to Castle.<sup>20</sup> Complete removal can be attained by repeated precipitation or by extraction with dilute acid or with alcohol. The active factor can be removed from beef muscles by repeated extractions with dilute acetic acid<sup>20</sup> or by extraction with 70 to 80 per cent alcohol.<sup>183</sup> It is water soluble, thermostabile, resistant to alkalization, readily soluble in 70 to 80 per cent alcohol but rapidly destroyed in 95 per cent alcohol.<sup>183</sup> The active substance is ultrafiltrable and cannot be extracted from an 80 per cent alcoholic solution with ether.<sup>183</sup> Following saturation of an alcoholic extract of beef muscle with ammonium sulfate the activity is found in the precipitate.<sup>183</sup>

The existence of an anemia due to a deficiency of Castle's extrinsic factor has been clearly demonstrated by Moore, Vilter, Minnich, and Spies.<sup>31</sup> These workers have described the occurrence of macrocytic anemia in 56 patients who had existed for years on diets inadequate in animal protein and in the vitamin B complex. Most of these subjects had at one time shown clinical evidence of pellagra, ariboflavinosis, or beriberi. The most striking clinical manifestations were weakness, pallor, glossitis, skin changes, and intermittent or persistent diarrhea. Eighteen of the patients showed signs of mild peripheral neuritis, but combined system disease was not observed. The anemia as well as the bone marrow was cytologically indistinguishable from true Addisonian pernicious anemia. The mean corpuscular volume ranged from 105 to 163 cubic microns and the mean corpuscular hemoglobin concentration from 27 to 38 per cent. Blood smears showed marked anisocytosis, poikilocytosis, polychromatophilia, and an occasional nucleated red cell. In most cases there was an associated neutropenia and thrombocytopenia. Nuclear hypersegmentation of both neutrophils and eosinophils was common. The bone marrow showed a shift to the younger forms of nucleated erythroid cells with many megaloblasts and early erythroblasts. Free hydrochloric acid was found in the gastric contents of 18 patients. In 5 of these the acid was present only intermittently. In 4 patients a persistent achlorhydria was demonstrated. All evidences of increased hemolysis were absent and the serum iron values were either low or in the normal range. Following the administration of an 80 per cent alcoholic extract of beef muscle a reticulocytosis developed and after prolonged administration the erythrocytes increased in number. All of the subjects showed a prompt therapeutic response to the parenteral injection of highly purified liver extracts. Studies on the pathogenesis of the anemia indicated that it was probably caused by a dietary deficiency of extrinsic factor associated in many, but not all, instances with poor absorption from the intestinal tract. Inadequate production of intrinsic factor was thought to be a complicating factor in some of the cases. Thiamine,

nicotinic acid, riboflavin, calcium pantothenate, pyridoxine, inositol, para-aminobenzoic acid and choline given together both orally and parenterally were ineffective in relieving the anemia. The anemia, leukopenia and thrombocytopenia all respond rapidly to pteroylglutamic (folic) acid therapy.<sup>643, 644</sup>

Wills reported the occurrence of macrocytic anemia of nutritional origin in India.<sup>97, 184-186</sup> The anemia is present in the lower classes which subsist on a low protein diet consisting principally of cereal grains. Although the blood and bone marrow pictures are indistinguishable from pernicious anemia there are several important differences. Nutritional macrocytic anemia is not accompanied by achlorhydria, the disease has an earlier age incidence, although it occurs equally in the two sexes, the anemia is frequently associated with pregnancy,<sup>187</sup> there is no evidence of increased blood destruction, there is an absence of neurological involvement, and the anemia fails to respond to the purified liver extract anahaemin which is so effective in the treatment of Addisonian pernicious anemia. The condition responds promptly to crude liver extracts such as campolon or to marmite or an autolyzed yeast extract. On the basis of the response to marmite, a substance which is ineffective in true Addisonian anemia, and its failure to respond to anahaemin Wills concluded that the anemia is due to a deficiency in the diet of some factor at present unidentified but other than Castle's extrinsic factor. Napier,<sup>188</sup> after studying nutritional macrocytic anemia in Calcutta, arrived at essentially the same conclusions except that, in addition, he found a group in which "anahamin" was effective; in these he felt that malaria was a predisposing factor. Fairley and Kondi<sup>190</sup> have reported the occurrence of a nutritional macrocytic anemia in Macedonia similar to that reported by Wills but differing in two important respects: the anemia was associated with a high indirect van den Bergh reaction and responded rapidly to "anahaemin." In a detailed description of nutritional macrocytic anemia in Macedonia Fairley, Bromfield, Foy, and Kondi<sup>191</sup> distinguished two distinct subgroups, hemolytic and nonhemolytic. Many of the cases were complicated, however, by malaria, splenomegaly and parasitism, and leukopenia and purpuric manifestations were often present. They concluded that the nonhemolytic cases were due to an uncomplicated dietary deficiency and suggested that in the hemolytic group chronic malaria supplied an additional hemolytic factor. Their cases responded slowly to large doses of marmite or to "campolon" injections. Fairley<sup>192</sup> has since reported an instance of this type of anemia in an Indian which responded satisfactorily to "anahaemin." Others have recorded a response to cruder preparations.<sup>193</sup> Rodriguez-Molina<sup>194</sup> has reported 2 cases occurring in Puerto Rico. One of these responded to purified liver extract and the other to marmite. Macrocytic anemia in Kenya has been reported by Anderson and Roberts.<sup>195</sup> Whether or not these anemias reported from various parts of the world are due to a deficiency of Castle's extrinsic factor cannot be determined from the confusing and conflicting evidence now available concerning their response to various liver fractions. It would be interesting to know if these tropical nutritional macrocytic anemias respond to the various pteroylglutamic acids.

Recently Watson and Castle<sup>196</sup> have made observations on 3 female patients

with nutritional macrocytic anemia occurring in the United States. These patients failed to respond to parenteral liver therapy but responded promptly to orally administered liver extract. They concluded that these patients had a deficiency of some substance other than the principle effective in pernicious anemia and that the "unitarian" hypothesis concerning the etiology of pernicious anemia and other nutritional macrocytic anemias did not apply to these patients. It is now known that this type of anemia responds to pteroylglutamic acid. This would be expected since these patients probably have a deficiency of folic acid rather than a faulty conjugase enzyme system as in true pernicious anemia.

Wills<sup>91-96, 197</sup> produced a macrocytic anemia with a megaloblastic marrow in monkeys by maintaining them on a diet based on one in common use among poorer class Mohammedans in Bombay, where nutritional macrocytic anemia is common. The diet consisted of polished rice, margarine, salt, iron, white bread, cod liver oil, and either tomatoes or carrots. Anemia of severe degree developed. Megaloblasts were constantly present in the peripheral blood at the height of the anemia and normoblasts were significantly increased. In all cases the van den Bergh tests were negative. The anemia responded rapidly with a marked reticulocytosis following the administration of marmite, or following campolon. "Anahaemin," a purified liver extract extremely potent in the treatment of pernicious anemia, was repeatedly ineffective. The active substance in marmite and campolon has been shown to be precipitated by mercuric acetate<sup>198</sup> and to be present in the soluble fraction after fractionation with ammonium sulfate. The insoluble fraction which is highly effective in pernicious anemia was ineffective in the treatment of the monkey anemia. From this Wills has concluded that the substance active against the experimental anemia is separate and distinct from the extrinsic factor of Castle. Both substances, however, have very similar characteristics. Both are water-soluble, soluble in dilute acetic acid, resistant to autoclaving, and soluble in 80 per cent alcohol but rapidly inactivated in 90 per cent alcohol. The relationship between the anemia produced by Wills and vitamin M deficiency is not clear. Morphologically they are reported to be quite different. The effect of folic acid on the monkey anemia of Wills has not been reported.

A macrocytic anemia has been produced in rats<sup>199</sup> and dogs<sup>200, 201</sup> but in both of these animals the anemia was complicated by Bartonella infection. The anemia which Rhoads and Miller<sup>202</sup> produced in swine was not definitely macrocytic as they claimed. Cartwright, Wintrobe, and Humphreys<sup>663</sup> maintained a single pig on a diet in which highly purified casein (supposedly lacking in extrinsic factor) was substituted for crude casein and to which 2 per cent sulfasuxidine was added. The animal failed to grow normally and developed partial alopecia and a normocytic anemia. Following treatment with a highly purified antipernicious anemia liver extract growth was resumed and the blood returned to normal.

F. Ascorbic Acid.- It has been conclusively shown that the scorbutic state in guinea pigs as well as in human beings is frequently accompanied by anemia. The nature and etiology of the anemia is obscure. The effect of pure ascorbic acid is in dispute.

1. *In animals.*- Meyer and McCormick<sup>205</sup> in 1928 reported the regular occur-

rence of anemia in experimental scurvy in guinea pigs. They suggested, without presenting much evidence, that the anemia was due to increased blood destruction. Mettier and Chew<sup>204</sup> reported reduction in hemoglobin to 6 to 8 Gm. per cent in guinea pig scurvy. Beginning 3 to 4 days prior to the death of the animals increasing numbers of reticulocytes appeared in the peripheral circulation. Examination of the blood smears revealed slight poikilocytosis, anisocytosis, polychromatophilia, and stippling. The bone marrows were hyperplastic with an increased number of normoblasts, suggesting a maturation arrest at this stage. Following the ingestion of 3 cc. of orange juice daily a reticulocytosis began usually on about the 3d day and reached the peak of production within 5 to 7 days. Following the reticulocyte response, there was an increase in the red blood cell and hemoglobin concentrations and a disappearance of the signs of scurvy. The bone marrows showed active and complete red cell maturation. Aron<sup>205</sup> maintained guinea pigs on a scorbutigenic diet supplemented with ascorbic acid for more than 50 days and no anemia developed. When the ascorbic acid supplement was withdrawn anemia developed. The addition of iron failed to prevent the development of anemia when the ascorbic acid was withdrawn. The anemia was quickly relieved in the less severe cases by the administration of ascorbic acid. In other instances ascorbic acid failed and germinated oats were required to relieve the anemia. Sigal<sup>206</sup> has demonstrated that the addition of ascorbic acid to a scorbutigenic diet prevents the development of anemia in the guinea pig. The morphological characteristics of this experimental anemia have not been carefully studied. Edel has reported that both the peripheral blood and the bone marrow show marked erythroblastosis.<sup>207, 208</sup>

2. *In human subjects.*—The frequent occurrence of anemia in human scurvy has been reported by many authors. Mettier et al.<sup>209</sup> state that in a large group of cases of scurvy in adults one may expect to find about one-third with red cell counts between 2 and 3 million per cubic millimeter, about one-third with red cell counts between 3 and 4 million, and the remainder with slight or no anemia. The red cells have been reported as being macrocytic, normocytic, and microcytic or hypochromic. McMillan and Inglis<sup>210</sup> in a study of 40 anemic scorbutic patients found macrocytic anemia in 2, normocytic anemia in 18, simple microcytic anemia in 14, and in 6 patients the anemia was microcytic hypochromic. Nucleated red blood cells were never seen and there was no reticulocytosis. The sternal marrows of 6 patients were examined. Five were normoblastic, 1 megaloblastic. Two of the former showed a few megaloblasts. The presence of megaloblasts was associated with achlorhydria in 2 instances and a low free HCl in another. One patient with a marked macrocytic anemia and normoblastic marrow had normal gastric secretion. As a result of various combinations of therapy they concluded that (1) the anemia observed was of nutritional origin, (2) ascorbic acid alone was not the cause of the anemia, (3) iron deficiency played a very minor part, (4) hemoglobin and red cell regeneration with reticulocytosis occurred in scorbutic patients on a vitamin C-free diet, and finally (5) that ascorbic acid was necessary in some deficient individuals before the anemia would respond to treatment. Parsons<sup>211, 212</sup> stated that in a series of 14 children suffering from scurvy 7 were anemic. The anemia was normocytic

normochromic in 3, normocytic hypochromic in 2, and macrocytic in 2. All of these patients failed to respond to iron but responded to the administration of ascorbic acid without any other alteration in the diet. Vilter and Woolford<sup>213</sup> studied 10 male adults with scurvy, 8 of whom had anemia. The red blood cell counts ranged from 1.74 to 3 million and the hemoglobin levels from 5.8 to 10.5 Gm. The cells were normocytic or moderately macrocytic. The reticulocytes fluctuated between 3 and 10 per cent and the icterus indices ranged from 10 to 22. The urine contained excess urobilinogen but no bile and the blood serum gave the indirect van den Bergh reaction in each instance. The bone marrows were of varying degrees of cellularity and in each case there was an increase in normoblasts. The anemia in all cases responded specifically to ascorbic acid. During the treatment period the patients were fed a diet free of vitamin C and low in the vitamin B complex. Gottlieb<sup>211</sup> reported 4 cases of "bachelor scurvy" associated with a high color-index anemia. In the 2 cases in which gastric analyses were done, hydrochloric acid was present in normal amounts. The patients were given a hospital diet plus ascorbic acid, the anemia responding satisfactorily. Jennings and Glazebrook<sup>215</sup> reported 2 cases of adult scurvy with anemia. In the first case there was definite macrocytic anemia as well as achlorhydria. Constant reticulocytosis, polychromasia, anisocytosis, and leukopenia were also observed. The bone marrow was hyperplastic with megaloblasts present. The anemia failed to respond to either liver or iron and was cured with ascorbic acid. The second case was characterized by normochromic normocytic anemia and megaloblastic bone marrow. A complete response was obtained with ascorbic acid. Mettier, Minot, and Townsend<sup>209</sup> found anemia in 8 of 9 cases of scurvy in adults. A normoblastic bone marrow was found in the 2 cases in which marrow examination was performed. The anemia responded to foods rich in vitamin C with prompt reticulocytosis and rapid regeneration of blood. Large doses of iron and liver had no effect. Others have reported that iron and liver preparations were useless and that the anemia responded to either pure ascorbic acid or to vitamin C-containing foods.<sup>216-219</sup>

There are several reports which fail to demonstrate a relationship between ascorbic acid and erythropoiesis. The most convincing of these is the report of Crandon, Lund, and Dill.<sup>220</sup> A normal active adult placed himself on a vitamin C-free diet supplemented with the other known vitamins for a period of 6 months. Although many manifestations of ascorbic acid deficiency appeared, anemia did not develop in spite of a blood loss from venesection of over 6 liters. Lozner<sup>221</sup> made observations in 5 patients with "presumptive vitamin C deficiency and anemia." In 4 of these, regeneration of hemoglobin took place spontaneously or in response to iron therapy alone. He concluded that "hemoglobin regeneration may occur in the absence of reduced ascorbic acid from the blood by chemical test." Actually only plasma ascorbic acid determinations were made. It is now generally recognized that a low level of vitamin C in the plasma does not necessarily indicate scurvy. It should be noted that one of Lozner's cases was complicated by a bleeding peptic ulcer, another by alcoholic pellagra, and a third was a woman with achylia, a urinary tract infection, and a history of nine pregnancies. Croft and Snorf<sup>222</sup> have been quoted as presenting evidence that synthetic vitamin

C is not the antianemic factor in scorbutic anemia.<sup>210, 221</sup> Actually the patients studied by them had none of the signs or symptoms of scurvy. The group consisted of 100 hospital patients taken indiscriminately who suffered from peptic ulcers, cirrhosis of the liver, infections, and a wide variety of other diseases. In all the ascorbic acid in the plasma was low. There is no reason to believe that the anemia which they treated unsuccessfully with ascorbic acid was due to scurvy. Other adequate and more likely causes for anemia were present.

Liu, Chu, Yu, Hsu, and Cheng<sup>223</sup> selected 16 anemic children from an institution. One-half were treated with ascorbic acid and one-half with ferrous carbonate. Eleven of the 16 cases according to the figures presented had macrocytic anemia. The iron-treated group responded with a marked rise in the erythrocyte count and hemoglobin although no reticulocytosis was noted. The group treated with ascorbic acid failed to respond. They concluded that the anemia was not due to a lack of vitamin C but related in all probability to a concomitant iron deficiency. It is strange that of the 8 patients reported as responding to iron only 1 had a microcytic anemia and 6 had macrocytic anemias. The absence of a reticulocytosis is also unusual. Whether there was increased capillary fragility in these cases is not clearly stated. Ralli and Sherry<sup>224</sup> maintained an anemic, scorbutic patient on a diet devoid of vitamin C but adequate in all other respects for 52 days. There was no further reduction in either the number of red cells or in the percentage of hemoglobin. Ascorbic acid therapy was discontinued on another patient after a response had occurred; after 45 days on a scorbutic diet no anemia appeared. Kenney and Rapoport<sup>216</sup> observed 3 scorbutic infants in whom there was no response to ascorbic acid but a good response to iron. Several authors have observed reticulocytosis and hemoglobin regeneration on a vitamin C-free diet.<sup>221, 225, 226</sup>

The literature dealing with the relationship of ascorbic acid to hemoglobin and red cell formation is difficult to evaluate. With the data available at the present time it would not be wise to draw dogmatic conclusions. Clinical scurvy is obviously complicated in many cases by multiple deficiencies, infection, and hemorrhage. It should not be expected that the blood picture would be uniform or that the therapeutic response to a certain substance would be constant in all cases. If several factors are deficient, the administration of only one of these should have little effect on red blood cell regeneration.

G. Other Vitamins.—1. *Pantothenic acid*.—The presence of a moderate normocytic anemia in dogs maintained on a diet deficient in filtrate factor II (pantothenic acid) was noted by Fouts, Helmer, and Lepkovsky.<sup>227</sup> Wintrobe et al.<sup>228</sup> noted a moderate normocytic, normochromic anemia in 13 of 18 pigs raised on a highly purified diet deficient in pantothenic acid. In 7 of the 13 animals the volume of packed red cells ranged between 35 and 40 cc. per 100 cc. of blood (normal 41 to 51). In the remaining 6 animals the volume of packed cells ranged between 24 and 33 cc. In the 2 animals receiving treatment with calcium pantothenate the anemia became less pronounced, the volume of packed red cells rising from 25 to 38 cc. in 1 animal and from 33 to 39 cc. per 100 cc. in the other.

It has been reported<sup>229</sup> that a deficiency of pantothenic acid in rats may result in

anemia, granulocytopenia, and bone marrow hypoplasia. These changes, however, were not produced in all of the animals. Although the inclusion of pantothenic acid in the diet almost completely prevented the appearance of anemia, it did not do so completely and therapy with this vitamin was even less successful once the deficiency was established. Some of the rats responded to *L. casei* therapy. It seems that a deficiency of pantothenic acid predisposed the animals to *L. casei* factor deficiency and that the prophylactic administration of pantothenic acid prevented the development of this complication. The therapeutic administration of pantothenic acid at times seemed to arrest the deficiency of *L. casei* factor. There was also evidence that instead of, or in addition to, *L. casei* factor deficiency there developed a deficiency of an unidentified vitamin.

2. *Choline*.—A marked decrease in hemoglobin and volume of packed red cells in dogs with a severe choline deficiency has been reported by McKibbin, Thayer, and Stare.<sup>230</sup> Treatment with choline failed in most cases to restore the blood entirely to normal but this may have been due to marked and irreversible damage to the liver.

3. *Biotin*.—Data have been presented by Ruegamer, Michaud, Elvehjem, and Hart<sup>231</sup> which indicate that biotin is necessary for the production of hemoglobin values greater than 14 grams per cent in dogs maintained on a highly purified alcohol extracted casein ration. Without biotin in the diet it was found that there was a plateau in hemoglobin values at 11 to 14 grams per cent and severe achromotrichia developed. No evidence that biotin deficiency in the rat has a significant effect in red blood cell or hemoglobin regeneration has been found.<sup>4</sup>

4. *Monkey anemia factor*.—Monkeys maintained on a purified diet supplemented with a folic acid concentrate and all of the known vitamins except riboflavin develop a hypochromic anemia and a leukopenia, as mentioned previously.<sup>6</sup> Upon the administration of riboflavin a definite increase in hemoglobin, red cell and white cell count in the blood has been observed but a plateau below normal is soon reached. Iron, "pseudo-pyridoxine," 1:20 liver powder, extracted liver residue, and increasing the casein level to 24 per cent have proved ineffective in restoring the blood picture to the normal level. A factor (or factors) found in whole liver substance was necessary for optimal blood regeneration. Thus, it would seem that monkeys require an additional factor for erythropoiesis. This factor (or factors) is evidently labile since commercial extraction procedures used to prepare 1:20 liver extract and extracted liver residue from whole liver destroyed nearly all activity. Fresh liver was found to be a more potent source than whole liver powder. Lyophilized liver retained all of the active principle of fresh liver.<sup>232</sup>

5. *Pigeon anemia factor*.—The presence of an anemia was noted in early experiments in which beriberi was produced by maintaining the birds on a diet of polished rice. Recent experiments using a purified diet have demonstrated that the anemia does not respond to thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, choline, para-aminobenzoic acid, and inositol.<sup>233-236</sup> The anemia does respond to yeast or crude liver extract. The possibility that the anemia is due to a deficiency of folic acid has not been ruled out although folic acid is adsorbable on fuller's

earth at pH 1, whereas the pigeon factor is very poorly adsorbed on fuller's earth at pH 4.0. Whether the dissimilarity in adsorption may be due to the difference in pH is not known.

6. *Guinea pig anemia factor*.—Severe anemia and leukopenia have been reported in guinea pigs fed succinylsulfathiazole and maintained on purified rations supplemented with the known vitamins, linseed oil meal, and solubilized liver.<sup>237</sup> Thus, there is convincing evidence that guinea pigs require an additional factor (or factors) for the maintenance of normal leukocyte and hemoglobin levels. This factor (or factors) is supplied in part by alfalfa, grass juice powder, crude liver extracts, and to a lesser extent by yeast. Folic acid does not appear to be the active substance since 4 per cent solubilized liver was included in the basal ration.

### III. AMINO ACIDS

It is understandable when one considers the relative size, complexity, and amino acid content of the globin fraction of the hemoglobin molecule that a dietary deficiency of protein or of a specific amino acid might result in anemia. Because of the difficulty in preparing a diet deficient in a single amino acid only a few experiments of such a nature have been conducted. These include deficiencies of tryptophan, lysine, and phenylalanine. Most of the work hitherto reported has been concerned with the rate of hemoglobin regeneration in animals made anemic by phlebotomy. Unfortunately, by this method it is not possible to study the characteristics of the anemia.

A. *General Protein Deficiency*.—Rats fed a diet abnormally low in protein but adequate in all other respects develop anemia which responds to protein therapy.<sup>238-241</sup> The anemia is characterized by a distinctly subnormal hemoglobin content of the blood, a normal erythrocyte count, and a mild reticulocytosis.<sup>241</sup> Metcalf, Favour, and Stare<sup>242</sup> have found that in acute protein deficiency in the rat there is an associated hemoconcentration and a significant decrease in the total circulating hemoglobin as the result of a reduction in the total blood volume. In chronic protein deficiency they found that although the hemoglobin concentration was unaltered the total circulating hemoglobin was significantly decreased. They point out that only if the total circulating hemoglobin is determined and adjusted to a unit of surface can the true severity of the anemia be appreciated. It has also been shown that dogs maintained on a diet deficient in protein show a progressive fall in the total red cell volume as well as in the red cell count and hemoglobin concentration.<sup>243</sup> The rate of blood regeneration in rats<sup>244, 245</sup> and in dogs<sup>246</sup> made anemic by repeated hemorrhage is accelerated by an adequate dietary intake of protein. Bethell<sup>247</sup> has suggested that the anemia which occurs in pregnant women and which is characterized by a normal color index and by red cells of normal or increased volume is due to a protein deficiency. Orten and Keller have found that the excretion of porphyrin in the feces of rats is diminished in animals fed a diet low in protein.<sup>248</sup>

B. *Composition of Globin*.—The minimal molecular weight of hemoglobin has been shown to be about 16,700 and studies of the osmotic pressure<sup>249, 250</sup> and of the sedimentation constant<sup>251, 252</sup> have shown that the true molecular weight of

anhydrous hemoglobin is close to 66,700, indicating that hemoglobin is composed of four globin units. According to Svedberg<sup>253</sup> globin is a polydisperse protein and does not have a definite molecular weight. There is evidence in the literature suggesting both the existence<sup>254-256</sup> and the absence<sup>257, 258</sup> of more than one hemoglobin in the same animal.

The amino acid composition of globin has been studied in several species and has been shown to contain all of the essential amino acids as well as many of the non-essential ones. It seems that the molecular ratios of tryptophan, tyrosine, arginine, histidine, and lysine are constant for all mammalian hemoglobins, being 2:3:3:8:9

TABLE 4.—*The Approximate Amino Acid Composition of Various Hemoglobins, in Per Cent*

Amino Acid	Horse	Man	Sheep	Bovine
Leucine.....	15.1 <sup>266</sup>	17.2 <sup>269</sup>	16.4-17.7 <sup>251</sup>	14-16 <sup>251</sup>
Isoleucine.....	1.6 <sup>260</sup>	0 <sup>267</sup>		
Histidine.....	7.7 <sup>265</sup>	8.0 <sup>265</sup>	7.4 <sup>265</sup>	6.8 <sup>265</sup>
Arginine.....	3.7 <sup>266</sup>	4.2 <sup>262</sup>	3.9 <sup>259</sup>	3.1 <sup>261</sup>
Lysine.....	8.6 <sup>266</sup>		7.7 <sup>261</sup>	8.0 <sup>261</sup>
Valine.....	9.8 <sup>264</sup>	10.1 <sup>261</sup>	7.7-8.1 <sup>264</sup>	7.5-10.3 <sup>264</sup>
Tryptophan.....	1.1 <sup>260</sup>		0.9 <sup>261</sup>	1.0 <sup>261</sup>
Phenylalanine.....	6.8 <sup>266</sup>		7.2 <sup>261</sup>	6.8 <sup>261</sup>
Methionine.....	0.75 <sup>262</sup>	1.2 <sup>262</sup>	1.2 <sup>262</sup>	1.3 <sup>262</sup>
Cystine.....	0.85 <sup>262</sup>	1.2 <sup>262</sup>	0.8 <sup>262</sup>	0.4 <sup>262</sup>
Threonine.....	6.8 <sup>268</sup>	6.8 <sup>260</sup>		
Tyrosine.....	3.0 <sup>266</sup>		2.2 <sup>261</sup>	2.0 <sup>261</sup>
Alanine.....	6.4-8.9 <sup>264</sup>	9.9 <sup>264</sup>	8.7-9.2 <sup>264</sup>	8.5-8.9 <sup>261</sup>
Glycine.....	5.6 <sup>266</sup>			
Serine.....	5.3 <sup>260</sup>			
Proline.....	2.0 <sup>260</sup>			
Hydroxyproline.....	0.0 <sup>260</sup>			
Aspartic Acid.....	10.3 <sup>266</sup>			
Glutamic Acid.....	8.5 <sup>266</sup>			5.6 <sup>260</sup>
Total Sulfur.....	0.4 <sup>262</sup>	0.6 <sup>262</sup>	0.6 <sup>262</sup>	0.4 <sup>262</sup>
Total Nitrogen.....	16.4 <sup>262</sup>	16.6 <sup>262</sup>	16.1 <sup>262</sup>	15.7 <sup>262</sup>

The superior numbers refer to the bibliographical source.

respectively, and that many of the differences in mammalian globins can be accounted for by the content of total sulfur, cystine, and methionine.<sup>261, 263</sup> The approximate percentage of amino acids in globin for several species is given in table 4.

C. Role of Amino Acids.—1. *General*.—The effects of the administration of various amino acids on the hemoglobin content of rats maintained on a low protein diet consisting of 3.5 per cent lactalbumin have been studied by Orten and Orten.<sup>269</sup> No consistent, sustained increase in hemoglobin values occurred following supplementation with any of the ten essential amino acids or with glycine, cystine, glutamic acid, proline, or tyrosine. They interpreted these results as evidence that no single amino acid can be regarded as a "key" amino acid in hemoglobin synthesis. It is logical to assume that if more than one amino acid is

lacking, then hemoglobin synthesis will not proceed until all of the missing amino acids are supplied. They conclude that "a combination of amino acids in as yet undetermined proportions is essential for the *in vivo* fabrication of the hemoglobin molecule."

Whipple and Robscheit-Robbins<sup>270, 271</sup> have shown that certain amino acids given to dogs made anemic by repeated hemorrhage cause an increase in new hemoglobin production over the basal level. Threonine, glycine, glutamic acid, aspartic acid, cystine, histidine, phenylalanine, and proline caused an increase in hemoglobin output of 23 to 24 grams above the control levels for a 2 week period. Leucine, methionine, lysine, and tryptophan caused an average increase of 20 grams and alanine, tyrosine, valine, isoleucine, arginine, and hydroxyproline increased hemoglobin output 10 to 17 grams over the control level for the 2 week period. There was no correlation between the quantity of an amino acid found in globin and its hemoglobin-regenerating activity.

In rats made anemic by the injection of phenylhydrazine Yashoda<sup>272</sup> found that histidine accelerated the formation of erythrocytes and hemoglobin and that tyrosine was ineffective in this capacity. Brand and Stucky<sup>273</sup> found that rats bred from mothers subsisting on a low protein (cystine-deficient) ration recovered from their "milk anemia" and showed good growth when glutamic acid was added to their iron supplement; whereas those from which glutamic acid was withheld but the iron added, died.

In dogs made both anemic and hypoproteinemic Whipple and his group<sup>271, 274</sup> have shown that globin can be readily formed from pure amino acid mixtures, plasma, serum digests, casein, hemoglobin given intraperitoneally, and hemoglobin digests given by mouth. The body seems to give preference to hemoglobin production over serum protein production.<sup>277-279</sup>

2. *Tryptophan*.—For some time it has been known that tryptophan accelerates recovery and causes a prompt reduction in the reticulocytosis seen in anemia caused by phenylhydrazine.<sup>280-283</sup>

By acid hydrolysis of casein it has been possible to prepare a protein which is almost totally deficient in tryptophan. This method has enabled workers to study the chemical and morphological characteristics of the anemia. Fontes and Thioville,<sup>284</sup> Hamada,<sup>282</sup> and Chin<sup>283</sup> reported that rats maintained on a diet in which the protein was supplied in the form of an acid hydrolysate of casein became anemic and that this anemia was relieved by the administration of tryptophan. Alcock<sup>285</sup> could not confirm these findings but Albanese et al.<sup>286</sup> have pointed out that Alcock's experiments were not of sufficiently long duration and that the diet may not have been entirely free of tryptophan. Albanese et al.<sup>286</sup> maintained rats on a diet in which the protein consisted entirely of acid hydrolyzed casein and reported that a mild normocytic, hypochromic anemia developed in 10 of 16 animals. The anemia in all cases responded to tryptophan therapy.

The anemia occurring in swine maintained on acid hydrolyzed casein as the only source of protein has been studied by Cartwright et al.<sup>57</sup> The anemia was normocytic, or slightly microcytic, and normochromic. It developed early and was slowly progressive. There was no evidence of increased hemolysis as determined

by icterus index and qualitative urobilinogen determinations. The serum iron levels remained within normal limits even at the height of the anemia and there was no reticulocytosis. Terminally, that is a week or so prior to death, leukopenia appeared and persisted in all 3 of the animals studied. Differential leukocyte counts revealed no consistent or significantly greater reduction in the number of cells of one series of leukocytes as compared with another. Blood platelets were not reduced in number. A hypoproteinemia developed simultaneously with the anemia. When the hypoproteinemia became severe peripheral edema appeared. The bone marrow in 2 of the animals was normoplastic and in the third hypoplastic. Hemosiderosis of the tissues was not present. The effect of adequate doses of tryptophan in restoring the animals to normal was not determined. Until sufficient tryptophan is available for this to be done it cannot be conclusively stated that the changes described were due to a deficiency of this amino acid.

As discussed earlier in this review pyridoxine-deficient rats, dogs, and swine excrete certain metabolites of tryptophan in the urine. In order to ascertain the effects of a low tryptophan intake on the course of pyridoxine deficiency Cartwright et al.<sup>57</sup> placed swine on a diet unsupplemented by pyridoxine and in which the protein was supplied in the form of acid hydrolyzed casein. It was concluded that a low intake of tryptophan retards the course and diminishes the severity of the nutritional disorder due to pyridoxine deficiency. This is in accord with the work of others in rats,<sup>53</sup> mice,<sup>53</sup> and dogs.<sup>54</sup>

Since a lack of either tryptophan or of pyridoxine leads to the development of anemia and since there is a disturbance of tryptophan metabolism in pyridoxine deficiency the question arises whether pyridoxine anemia may be due to a lack of properly metabolized tryptophan. If such were true the two anemias should be similar. They are, however, quite different. The anemia of pyridoxine deficiency is microcytic and slightly hypochromic and is accompanied by an elevated serum iron, hyperplastic bone marrow, and hemosiderosis of the liver, spleen, and bone marrow. The anemia associated with the feeding of acid hydrolyzed casein is essentially normocytic and normochromic, the bone marrow appears to be hypoplastic, the serum iron level is normal and there is no hemosiderosis of the tissues. The two deficiencies are markedly different. Tryptophan deficiency causes cessation of growth and is accompanied by marked hypoproteinemia and edema. Pyridoxine deficiency causes only limitation of growth and the quality and quantity of the serum proteins are unaffected. The two anemias are compared in table 5.

3. *Lysine*.—It is known that deaminization of casein destroys the entire content of lysine, about half of the histidine, and a portion of tyrosine.<sup>257</sup> Hogan and Ritchie<sup>288</sup> reported that rats maintained on deaminized casein develop anemia and splenomegaly. When casein was added to the deaminized casein ration the anemia failed to develop. Smith and Stohlman<sup>289</sup> studied the anemia and reported that after 10 to 15 days on the diet examination of the blood revealed anisocytosis, a moderate degree of reticulocytosis, polychromatic macrocytes frequently containing one or more Howell-Jolly bodies, and a substantial degree of anemia. As the disease progressed the anemia became more pronounced, the red cells falling as low as 2 million and the hemoglobin to 20 per cent. The blood smears in the

advanced stage showed many poikilocytes, microcytes, macrocytes, polychromatic cells, "megaloblasts," and numerous Howell-Jolly bodies. The reticulocyte count ranged from 5 to 25 per cent. Immature white cells such as myelocytes and myeloblasts appeared in various numbers. The anemia was usually macrocytic and slightly "hyperchromic." By boiling deaminized casein with alcoholic sodium hydroxide, or by reprecipitating the deaminized casein from aqueous alkaline solution, the anemia-producing factor could be destroyed to a considerable extent. The intraperitoneal injection of the alcohol-soluble fraction of a hydrochloric acid hydrolysate of deaminized casein reproduced the anemia and the authors concluded that the anemia was not a deficiency disease but was due to the presence of a toxic substance in the deaminized casein. Hogan and co-workers<sup>290-292</sup> extended their experiments and found that lysine was the antianemic agent in the deaminized casein-anemia syndrome. However, it was necessary to administer 2 to 4 times the

TABLE 5.—*A Comparison of the Anemias Produced by Casein Hydrolysate and Pyridoxine Deficiency in Swine*

Determination	Casein Hydrolysate	Pyridoxine Deficient	Control
R.B.C., millions cu. mm. . . . .	4.58	6.50	7.93
HBG. Gm. % . . . . .	8.2	8.9	14.0
Hematocrit, cc. . . . .	24.0	29.3	46
MCV, $\mu$ m . . . . .	52	41	58
MCH, $\mu$ g . . . . .	18	14	18
MCHC, % . . . . .	34	30	33
W.B.C., cu. mm. . . . .	5500	17,500	18,000
Serum Iron, $\mu$ g % . . . . .	131	374	142
Total Protein, Gm. % . . . . .	3.88	6.27	6.33
Bone Marrow . . . . .	N	H	N
Hemosiderosis . . . . .	0	+	0

N = Normoplastic.

H = Hyperplastic.

normal requirement of lysine to prevent the anemia. These authors speculated that lysine was required to detoxify the deaminized casein. Attempts to isolate lysine from the urine of rats supplied with this amino acid failed.

Gillespie, Neuberger, and Webster<sup>293, 294</sup> have presented evidence that lysine deficiency in rats results in an anemia. They maintained rats on a diet in which the protein was supplied in the form of zein, a protein deficient in both lysine and tryptophan. One group was given a daily supplement of tryptophan and lysine. A second group received only tryptophan. In the group receiving lysine the average red cell count and hemoglobin were 8.76 million and 14.9 Gm. per cent, respectively. In the group receiving no lysine the average red cell count was 7.10 million and the average hemoglobin 11.1 Gm. per cent. Thus there was a mild anemia with a slightly greater average reduction in hemoglobin than red cells. In a third group of animals it was demonstrated that the anemia was not due to inanition.

Muller<sup>295</sup> has reported that when lysine is injected intravenously into pigeons a

reticulocytosis occurs, this reticulocytosis being caused by a stimulation and proliferation of the red blood cells in the bone marrow with an extension of the blood-forming marrow tissue.

4. *Phenylalanine*.—Maun, Cahill, and Davis<sup>256</sup> fed synthetic diets of crystalline amino acids, crystalline vitamins, fats, dextrin, and salts to young rats. When phenylalanine was omitted from the amino acid mixture for 28 days the hemoglobin ranged from 7.4 to 14.0 Gm. per cent, with an average of 9.9 Gm.; animals on the complete diet had hemoglobin levels ranging from 13.7 to 15.8 Gm., with an average of 14.7 Gm. Hemosiderosis of the tissues was not noted.

5. *Glycine*.—Evidence for the direct utilization of glycine for the formation of the pyrrole rings of protoporphyrin in humans has been presented by Shemin and Rittenberg.<sup>257</sup> Glycine containing  $N^{15}$  was consumed by a human subject. Hemin was then isolated at various intervals and found to contain significant quantities of  $N^{15}$ . Previous experiments in which leucine<sup>258</sup> and ammonia<sup>259</sup> labeled by  $N^{15}$  were fed to rats gave no indication that these substances were directly concerned with porphyrin synthesis. It has been suggested that tryptophan, proline, and hydroxyproline are precursors of protoporphyrin. There is no direct evidence to confirm this.

6. *Isoleucine*.—Orten, Bourque, and Orten<sup>260</sup> have presented evidence that isoleucine deficiency in the rat results in a mild to severe anemia. The animals were fed a synthetic diet containing purified human or beef globin as the protein. Both of these proteins have been shown to be deficient in isoleucine. Supplementation of either type of globin with isoleucine resulted in the maintenance of a normal concentration of hemoglobin in the blood. The subsequent removal of isoleucine was followed by the development of anemia and death of the animals.

The finding that isoleucine is needed for hemoglobin formation in the rat raises an interesting question. Either the globin of rat hemoglobin, unlike that of human or beef hemoglobin, contains isoleucine or isoleucine must be concerned in the formation of some intermediate compound.

#### IV. MINERALS

A. *Iron*.—That iron is a constituent of the hemoglobin molecule<sup>301, 302, 304</sup> and that a deficiency of iron gives rise to an anemia are both well established. The magnitude of the literature on various aspects of iron metabolism is great, yet there are large gaps in our knowledge of certain phases. In recent years there have been several reviews on the subject.<sup>303-311</sup> However, since these reviews were published the fundamental concepts of iron metabolism have changed greatly.

1. *Availability*.—Dietary iron is of two types, organic and inorganic.<sup>312</sup> The organic iron is present principally in the form of iron porphyrin compounds. The ability of inorganic iron salts to cure iron deficiency anemia has been well demonstrated in many different species. On the other hand, it has been shown that organically bound iron is very poorly utilized when given orally. Bunge's theory<sup>313</sup> that iron occurs in food as complicated organic compounds which are absorbed and assimilated as such and are built directly into hemoglobin has not been confirmed by modern studies. Organic iron, if it is to become available, must be

converted to an ionizable form. In the case of hematin it has been shown that it is less than 25 per cent utilized and this utilization appears to be dependent upon the amount of decomposition by intestinal bacteria.<sup>314, 315</sup> It has not been demonstrated that a preformed iron compound is absorbed and used as such in building the hemoglobin molecule. On the available information it would seem that the body's need for iron can be entirely satisfied by inorganic iron compounds.

Hill<sup>316</sup> has shown that  $\alpha$ -dipyridyl reacts with ionizable ferrous iron to form an intense red compound. The reagent does not react with hematin or other organically bound iron compounds. Elvehjem and co-workers<sup>317, 318</sup> have shown that there is a moderately good correlation between the biologically active iron in foodstuffs and the iron determined with  $\alpha$ -dipyridyl following reduction of the ferric iron. Tables of the "available" iron content of foodstuffs have been made using this method. Their value is somewhat limited for as Hahn and Whipple<sup>319</sup> pointed out, the term "available iron" as determined by the dipyridyl test has little physiological significance. An iron salt which is rated as 100 per cent available by the dipyridyl test may be only 40 per cent physiologically active.

2. *Absorption*.—Iron is absorbed chiefly in the duodenum, but the stomach and the whole of the small intestine may take part under certain circumstances.<sup>320-322</sup> It is doubtful that any absorption takes place from the colon.<sup>323</sup> It is absorbed by the tips of the villi of parts of the duodenum and from there is passed into the portal circulation and is carried to the liver. Moore et al.<sup>324</sup> have shown that iron is not transported by the thoracic duct lymph.

There are many factors affecting the absorption of iron. By measuring the serum iron increase following the oral administration of iron in various forms and under various conditions Moore and co-workers<sup>322, 324</sup> could define many of these factors. Whipple and his group, working with an entirely different method, have been able to define others. The present knowledge concerning the absorption of iron may be summarized as follows:

a. *Type of iron*.—It is generally believed that iron is absorbed in the soluble, ionizable, ultrafiltrable, ferrous form. The absorption of ferric compounds is generally less than that of ferrous compounds and is dependent on the capacity of the intestinal contents to reduce them. The nature of the anion, except as it influences the ease of ionization, is relatively unimportant. Organically bound iron as stated above is very poorly absorbed.

In the past there has been disagreement between the clinical and animal investigators as to whether the ferrous form is more readily absorbed than ferric iron. Using the radioactive iron technic it has been demonstrated in two different laboratories that human subjects absorb ferrous iron more efficiently than ferric iron.<sup>325, 326</sup> Dogs absorb both valence forms well although in some instances there is a greater uptake of ferrous than ferric iron. Rats absorb radioactive ferrous and ferric iron equally well.<sup>327</sup> Thus there is a species difference and this probably accounts for the disagreement between clinical and animal investigations. Moore et al.<sup>325</sup> offer three possible explanations for the greater absorption of bivalent iron for the human being: (1) only ferrous iron may be absorbed and all trivalent iron may have to be reduced before it can be taken up by the body; (2) both forms

may be absorbed but to an unequal degree; and (3) ferric iron may be made less available for absorption because it more readily forms complex insoluble compounds within the intestinal tract.

b. *Amount of iron*.—The height of the serum iron increase is directly proportional to the amount given up to that point at which intestinal irritation is great enough to interfere materially with intestinal motility.

c. *Gastric acidity*.—The free gastric hydrochloric acid performs two functions: (1) reducing, ionizing, and dissolving the iron, and (2) delaying the formation of insoluble and undissociated iron compounds which may occur above pH 5. For these reasons any factors which cause increased alkalinity diminish iron absorption.

d. *Conditions in the duodenum*.—The pH of the duodenal contents likewise affects the ionization and solubility of the iron compounds. The presence of certain reducing substances in the diet aids the absorption of iron. Fisher and Peabody<sup>328</sup> have shown that liver extract has marked reducing properties. Oxidation of ferrous iron is effectively prevented by liver extract and 50 to 95 per cent of ferric iron added to it is reduced. The reducing properties of ascorbic acid are well known.<sup>324</sup> Freeman and Ivy<sup>329</sup> have shown that calcium carbonate, aluminum hydroxide, and to a lesser extent magnesium trisilicate reduce iron retention in anemic rats. The absorption of iron from the intestinal tract is inhibited when iron is administered with relatively large doses of mucin in cases of chronic hypochromic anemia.<sup>330</sup>

e. *Ca:P ratio and vitamin D*.—There is ample evidence that excess calcium in the diet inhibits iron assimilation and causes a mild anemia.<sup>331-336</sup> Furthermore, all investigators are agreed that iron utilization is affected by the Ca:P ratio in the diet but there is disagreement as to whether a high or low ratio is more favorable. Anderson, McDonough, and Elvehjem<sup>334</sup> have shown that hemoglobin regeneration and iron storage in the liver were the greatest on the lowest Ca:P ratio and that, as the ratio was increased, there was a corresponding decrease in both hemoglobin regeneration and iron storage. These results have been confirmed by Fuhr and Steenbock<sup>335, 336</sup> but are contradictory to those of Day and Stein.<sup>337</sup> The latter workers found that rats on a low mineral ration containing a relative excess of phosphorus develop a mild numerical increase in red cells together with a reduction of hemoglobin and that this is prevented by increasing the calcium in the diet. The action of calcium is explained by its ability to bind phosphorus, "thus permitting the dietary iron to be used for hematopoiesis instead of being excreted, presumably as a phosphate."

Where the discrepancies lie between these two experiments is not clear but the important fact is that neither a high nor a low Ca:P ratio is incompatible with normal hematopoiesis if the diet contains sufficient iron, for the action of these minerals is not directly on hemoglobin formation but rather on iron absorption. When this is appreciated the anemia accompanying rickets is better understood.

Fuhr and Steenbock<sup>338</sup> have demonstrated in rats that vitamin D improves the storage of iron and the formation of hemoglobin when the diet contains optimal amounts of calcium and phosphorus. A similar stimulus to hemoglobin formation was noted by Day and Stein.<sup>337</sup>

f. *Selective absorption*.—McCance and Widdowson<sup>339-341</sup> introduced the idea that the intestine controls the amount of iron absorbed. Robscheit-Robbins and Whipple<sup>342, 343</sup> have shown that the efficacy of iron feeding depends upon the actual need of the body for the element. Recent studies by Whipple and co-workers<sup>344</sup> using a radioactive isotope of iron have been enlightening. They showed that dogs made anemic by frequent bleeding while on a low iron diet had 4.1 to 12.7 per cent of the ingested radioactive iron in the tissues and blood while normal nonanemic dogs had only 0.08 to 0.24 per cent accountable. These experiments were extended<sup>323</sup> and it was concluded that the mucosa of the gastrointestinal tract has the power to reject or to accept iron. These studies were then carried to human beings<sup>345</sup> and it was found that when there is need of the body for iron as in pregnancy and iron deficiency states, the iron intake is increased above normal, whereas in disease states in which the iron stores are known to be very abundant as in pernicious anemia, hemochromatosis, familial hemolytic icterus, and Mediterranean anemia, there is very little absorption. They concluded that "reserve stores of iron in the body, rather than anemia, control iron absorption. This control is exerted on the gastrointestinal mucosa which can refuse or accept iron under various conditions."

A possible fallacy in the experiments of Hahn and Whipple on the absorption of iron in various anemic states in man must be pointed out. The amount of radioactive iron appearing in the red cells was used as an indication of the amount absorbed. In anemic states other than iron deficiency it is possible and even likely that owing to a diminished rate of hemoglobin formation much of the absorbed iron does not appear in the hemoglobin. The amount of iron appearing in the hemoglobin is not necessarily an indication of the amount absorbed but rather of the amount utilized. If hemoglobin cannot be formed in cases in which iron is not the limiting factor then iron, even though absorbed, cannot be utilized. In such cases the amount of iron appearing in the red cells would give a false impression of the amount absorbed.

Subsequent studies by Hahn and co-workers<sup>321</sup> as to the precise mechanism by which the gastrointestinal mucosa accepts and refuses iron have revealed that an acute severe anemia or anoxia of 24 hours' duration does not significantly increase iron absorption. After approximately 7 days, at which time the iron stores are depleted, absorption becomes active. These workers speculate that there is an "acceptor compound" in the mucosa which is capable of taking up iron and passing it on to the plasma, and that the plasma iron concentration may regulate the degree of iron saturation of this acceptor and consequently its ability to pass iron from the intestinal lumen to the plasma. They further speculate that once iron reaches the plasma it is combined with protein in a combination which is not sufficiently loose to allow transfer of the iron in the reverse direction through the acceptor. "Thus this acceptor together with the action of the plasma might act as a valve mechanism allowing the body to obtain iron when needed and conserve what it has for future needs."

Granick<sup>302, 664, 665</sup> has discovered that the content of ferritin in the gastrointestinal mucosa is relatively high and that it increases in response to iron feeding.

On this basis he has suggested the following modification of the hypothesis of Hahn et al.: Iron is absorbed into the mucosal cells in the ferrous form where it is stored as ferritin (a combination of the protein apoferritin with ferric hydroxide). There is an equilibrium in the cell between ferrous iron and the ferric iron stored in ferritin, the cell being in a state of "physiological saturation" with respect to ferrous iron. As the concentration of the plasma iron falls, ferrous iron is removed from the mucosal cell, resulting in a diminution of ferritin iron in the mucosa. When the ferritin iron has diminished to a point where the cell is no longer saturated with respect to ferrous iron, more iron is absorbed by the cell.

Moore and his group<sup>322</sup> have questioned the selective absorption theory and cite as evidence against this theory the fact that some patients with hypochromic anemia before therapy do not have greater iron absorption curves than after therapy. They believe that a more likely explanation would be that iron is absorbed by a simple process of diffusion through the interepithelial spaces into the blood stream and that the magnitude of the concentration of the gradient of iron between the intestinal lumen and blood plasma determines the amount of iron absorbed. As pointed out by Hahn et al., this theory does not explain the fact that smaller doses of iron are more efficiently absorbed than larger ones. And as noted by Moore et al., it is in contrast to the histological experiments of Höber<sup>316</sup> which tend to show that iron is absorbed intra-epithelially.

Since Moore et al. admit that iron-deficient animals absorb iron more readily than those with adequate stores and that there is no histological evidence for simple diffusion through the interepithelial spaces, and since Hahn et al. now believe that the serum iron level may play a role, it would seem that the divergence between these two views is not great.

*g. Other factors.*—There is suggestive evidence that pyridoxine may regulate iron absorption. This has been discussed under pyridoxine. It has been shown that the average retention of iron in children is slightly lower during periods of high thiamine intake than during control periods.<sup>317</sup> These results, however, could not be confirmed in rats on a well controlled experiment.<sup>318</sup> The amount of food consumed affects iron absorption. With a constant intake of iron, an increase in consumption of food results in a lower retention of iron, and a decrease results in a higher retention.<sup>347</sup>

*3. Transportation.*—There is now ample experimental evidence that there is a fraction of iron in the plasma which is in a nonhemoglobinous form.<sup>323, 349–351</sup> Using radioactive iron Yoshikawa, Hahn, and Bale<sup>355</sup> have shown that about 95 per cent of the plasma iron fraction is bound to protein and of this only 15 per cent is bound to the globulin fraction. However, 90 per cent of the plasma iron is not precipitated by trichloroacetic acid and therefore must be very weakly bound. Whether there is true salt formation or whether the protein tie-up is one of adsorption has not been determined. *In vitro* studies have shown the serum iron is in the ferric state and as such is nonultrafiltrable but upon reduction to the ferrous state becomes ultrafiltrable.<sup>356</sup> This is known as the "Barkan phenomenon." The true state of plasma iron *in vivo* is unknown.

The significant fact concerning plasma iron is that it has been demonstrated

to function as transport iron.<sup>323, 350</sup> Following a large dose of iron salts there is a prompt rise in the plasma iron fraction. The increase is apparent within the first half hour, reaches its maximum in  $2\frac{1}{2}$  to 5 hours, and falls to the basal level in 12 to 18 hours. It is interesting that transportation and utilization are very rapid processes. Radioactive iron has been shown to find its way into red cells in as little time as several hours.<sup>323</sup> No direct interchange between the plasma iron and the red cell has been demonstrated.<sup>357</sup>

The normal range for plasma iron in human beings has been shown to be about 0.050 mg. to 0.180 mg. per cent.<sup>349</sup> The values obtained by various workers are given in table 6. The average value for men is slightly higher than for women. There is essentially no difference between the amount contained in plasma as compared with serum.<sup>349</sup> Moore, Minnich, and Welch<sup>354</sup> found that the difference between minimum and maximum serum iron values in a given normal subject

TABLE 6  
*The Serum Iron of Normal Subjects as Obtained by Various Investigators (Modified from J. A. Powell<sup>350</sup>)*

Author	Men			Women			Difference D $\pm$ $\epsilon$ D
	No. of Ob- ser- va- tions	M $\pm$ $\epsilon$ M	$\sigma$	No. of Ob- ser- va- tions	M $\pm$ $\epsilon$ M	$\sigma$	
Heilmeyer and Plötner <sup>351</sup> .....	25	126.2 $\pm$ 4.3	21.4	25	88.5 $\pm$ 3.8	18.8	+37.7 $\pm$ 5.1
Moore, Arrowsmith, Quilligan, and Read <sup>349</sup> ..	15	121.5 $\pm$ 6.7	25.8	15	97.6 $\pm$ 6.1	23.7	+23.9 $\pm$ 9.1
Vahlquist <sup>359</sup> .....	50	142.0 $\pm$ 6.1	43.0	50	123.0 $\pm$ 4.5	31.6	+19.0 $\pm$ 7.6
Powell <sup>350</sup> .....	35	143.0 $\pm$ 4.1	24.0	35	117.0 $\pm$ 4.5	26.5	+26.0 $\pm$ 6.1

M = Mean;  $\epsilon$ M = Mean error of mean; D = Difference;  $\epsilon$ D = Mean error of difference;  $\sigma$  = Standard deviation. The superior numbers refer to bibliographical source.

ranged within the daily cycle and during a 6 month period from 0.01 to 0.065 mg. per cent. Heilmeyer and Plötner<sup>358</sup> have found variations of -10 to +19 micrograms per cent in 6 normal subjects during a period of 1 to 9 days. Similar observations have been made by others.<sup>361, 362</sup> Hemmeler<sup>363</sup> has presented evidence that the serum iron is highest early in the morning, decreases gradually during the day, and rises during the night. Vahlquist<sup>362</sup> found higher values at 6 P.M. than at 8 A.M. Evidence has been obtained for a menstrual periodicity in the serum iron in normal women.<sup>360</sup> The serum iron has been found to be lower in old people than in young.<sup>364</sup> There is evidence that physical exercise decreases the serum iron.<sup>365</sup> Reginster<sup>366</sup> induced fever in 3 subjects and noted a lowering of the plasma iron level.

The plasma iron level is affected by the rate of absorption of iron, the balance between that going to and from the tissues, and the equilibrium between the amount used for hemoglobin formation and that coming from hemoglobin catabolism. The values found in various pathological states are given in table 7. In general, in iron deficiency states it is uniformly low. Under conditions of decreased red cell formation (aplastic anemia, Mediterranean anemia, myelophthisic anemia,

and pyridoxine deficiency anemia in animals) it tends to be high. When the bone marrow is unusually active (acute hemorrhage and liver-induced remission in pernicious anemia) the plasma iron is low. In hemolytic states the plasma iron level is dependent upon the equilibrium between the iron released by hemolysis of red cells and the rate of uptake by the bone marrow.

Barkan<sup>375-378</sup> has described a third form of blood iron, constituting 5 to 10 per cent of the total blood iron, which he termed the "leicht abspaltbare Bluteisen" fraction. This was so named because it was easily freed from its lightly bound state in the erythrocytes and plasma by acidification with weak acids. This finding has since been many times confirmed.<sup>379</sup> When whole blood is incubated with 0.1 N hydrochloric acid 2.3 to 2.5 mg. per cent of iron is "easily split-off."<sup>349</sup> Sulfuric and nitric acids split off more iron than does hydrochloric acid.<sup>349</sup> The concentration of the acid also affects the amount of "easily split-off" iron ob-

TABLE 7.—*Showing the Plasma Iron Level in Various Conditions in Human Beings*

Condition	Plasma Iron	Reference
Iron Deficiency....	Low	72, 350, 358, 360, 372
Mediterranean Anemia .	High	72
Familial Elliptocytosis ..	High	367
Pernicious Anemia....	High	31, 72, 350, 360
Nutritional Macrocytic Anemia...	Normal or Low	31, 72
Sprue.....	Low	72, 369
Aplastic Anemia..	High	72, 350, 360
Infections.....	Low	370
Tuberculosis.....	Low	366, 371
Myelophthisic Anemia.	High	350
Nephritis ...	Variable	72, 350
Malaria.....	Variable	72
Hemolytic Anemias ..	Variable	350, 360
Acute Hepatitis...	High	373, 374
Hypothyroidism..	Normal	350
Hemochromatosis. .	Normal	350

tained.<sup>349</sup> There is no significant change in the amount of iron split off with concentrations of hydrochloric acid between 12 and 2 per cent, but there is a definite increase below this concentration with a sharp peak at about 0.1 per cent.<sup>379</sup> Moore and his group have clearly demonstrated that the "leicht abspaltbare Bluteisen" fraction does not function as transport iron<sup>351</sup> as was originally suggested by Barkan.

The "leicht abspaltbare Bluteisen" fraction was subdivided by Barkan into two parts, E and E'. E was that portion present only in red cells, constituting 60 to 70 per cent of the total, and bound by carbon monoxide in such a manner that it was protected against the "splitting-off" action of weak acids. E' was that fraction which was present in the plasma as well as the red cells, was not bound by carbon monoxide, and constituted 30 to 40 per cent of the total. The whole of the plasma iron was included in this fraction. Barkan advanced the view that the "easily

split-off" fraction constituted an organic, nonhemoglobinous form of iron and finally succeeded in establishing that the iron of iron-containing bile pigment compounds (pseudohemoglobins, verdohemochromogens), that is, open ring derivatives of hemoglobin, was "easily split off" by the action of dilute acids.<sup>378, 380</sup> He concluded from this that the "easily split-off" iron was due to the presence in the erythrocytes of verdohemochromogens. It has since been shown by Legge and Lemberg,<sup>381</sup> and confirmed by Barkan and Schales<sup>379</sup> and others,<sup>382</sup> that the E fraction is an artefact arising from oxidation of the prosthetic group of the hemoglobin molecule by the O<sub>2</sub> evolved from oxyhemoglobin by acids. The E' fraction is partially attributable to a bile pigment-hemoglobin, a small part arises from blood catalase, and the remainder is due to the iron in the plasma.

TABLE 8.—*The Distribution of Iron in the Body as Determined in a Dog Weighing 20 Kilograms (Modified after Hahn<sup>306</sup>)*

	Per Cent Total Body Iron
Hemoglobin iron	57
Myoglobin iron	7
Parenchyma iron cytochrome catalase peroxidase	16
Storage iron ferritin ? noncrystallizable ferritins ? ferrin hemosiderin Inorganic ferrous iron	20

4. *Storage of iron.*—Although the literature on this phase of iron metabolism is very extensive the storage of iron is poorly understood. There are numerous studies of the iron content of tissues as found under various conditions but little information is available concerning the chemical nature or physiological function of the various fractions. The exact total iron content of a specific tissue is dependent on many factors and is relatively unimportant. Many of the studies reported in the literature were done on tissues which were not blood free and with methods which have since been shown to be untrustworthy.<sup>383</sup> The work of Whipple and his co-workers<sup>384, 385</sup> on the total iron content of tissues under various circumstances has been carefully done on blood-free tissues and with reliable methods and only this work will be summarized here. Subsequent papers confirm these findings but add little further knowledge on this phase of iron metabolism.

Present knowledge concerning the distribution of iron in the body of a dog of 20 Kg. weight (modified from Hahn<sup>306</sup>) is summarized in table 8.

The immobile fraction of body iron is made up of parenchyma iron and the iron

in myoglobin. This constitutes about 23 per cent of the total body iron. The Rochester group have shown that in the face of long-continued severe anemia and urgent need for iron from any available source, the dog does not draw on either the parenchyma iron of the tissues or on the muscle hemoglobin iron. Parenchyma iron is probably held tightly by the cells in such forms as cytochrome, catalase, peroxidase, and other cellular enzymes and is essential to the life of these cells. Myoglobin has been shown to be very similar to hemoglobin in respect to absorption,<sup>386</sup> iron content,<sup>387, 388</sup> and elimination by way of bile pigments<sup>389</sup> but may be differentiated by means of a specific precipitin reaction.<sup>390</sup>

Whipple and his co-workers established the location of stored iron by injecting iron in dogs which had been made iron deficient for 2 to 3 months through repeated phlebotomy and a low iron diet. From these studies they concluded that the liver, spleen, and bone marrow are the principal sites of storage iron. Fifty to 70 per cent of iron injected intravenously can be shown to be stored in the liver and spleen. The liver contains the main bulk of the iron stored and is considered the most important organ in the conservation and utilization of iron in the body. The turnover in the liver is exceedingly rapid if there is a demand for the element for blood formation. However, the spleen contains the highest reserve stores of iron per 100 Gm. of fresh tissue. The iron content of the rib bone marrow runs parallel with that of the spleen as judged by iron storage following hemoglobin injections and depletions. There is some evidence that the manner in which iron arrives at the tissues affects its distribution.<sup>391</sup> When radioactive ferric ammonium citrate was given intravenously most of it appeared in the liver. Iron liberated from hemoglobin by destruction of the red cells with phenylhydrazine was taken up by the spleen as well as by the liver. It has been shown that the kidney forms an important function in the conservation of iron.<sup>391</sup> The glomeruli establish the minimal renal threshold for hemoglobin and prevent its escape. When this threshold is exceeded the tubular epithelium picks up the hemoglobin and saves it for future use. Finally when the tubular threshold is exceeded hemoglobin appears in the urine.

By use of the radioactive isotope it has been demonstrated that the normal stores of iron are not the first line of supply for blood formation when massive hemoglobin destruction has occurred.<sup>392, 393</sup> Rather the hemoglobin iron of the new cells is first derived from the cells broken down. The stores are drawn upon only if additional iron is necessary as a result of abnormal loss of the element from the body.

*(To be continued in the next issue)*

# THE EFFECT OF DIET ON THE HEMOGLOBIN, ERYTHROCYTE, AND LEUKOCYTE CONTENT OF THE BLOOD OF THE RHESUS MONKEY (*MACACA MULATTA*)

By EDITH SEYMOUR JONES, KEITH B. McCALL, C. A. ELVERJEM, PH.D.,  
AND P. F. CLARK, PH.D.

**S**TUDIES on the nutritional requirements of the rhesus monkey (*Macaca mulatta*) have been in progress in our laboratory during the past five years. The major work has been concerned with the B group of vitamins and routine blood examinations were made as each vitamin deficiency was studied. Previous reports from this and other laboratories have clearly shown the significance of certain nutritional factors in the maintenance of normal blood constituents. As the exact nutritional requirements of the monkey are more clearly understood now than at the time of the initiation of these experiments, it is possible to determine more accurately how a deficiency of several of the B vitamins adversely affects the concentration of certain blood constituents.

The literature contains about twenty reports regarding the blood picture of the normal rhesus monkey. Shukers, Langston, and Day<sup>1</sup> and Suarez, Rivera, and Morales<sup>2</sup> have summarized the greater part of the reports. Scarborough<sup>3</sup> has compiled the work preceding 1926 including the earliest, that of Hayem.<sup>4</sup> Values obtained by these and other workers are presented in table 1. It is evident from these reports that inadequate consideration was given to the nutritional status of many of the experimental animals. The values reported often showed extreme ranges and consequently the arithmetic average was of questionable value. Although Krumbhaar and Musser<sup>5</sup> controlled conditions such as food, standard time of bleeding, and selection of animals as to size and age, they still obtained variations greater than those attributable to experimental error.

As a result of our nutritional studies we have found that when monkeys are fed the basal diet M-3<sup>21</sup> supplemented with vitamins and whole liver substance, the concentration of certain blood constituents is somewhat different from those previously accepted as normal values. In this paper we wish to report the blood picture of rhesus monkeys receiving purified rations supplemented with vitamins and various liver products and to summarize our data regarding the effect of certain vitamin deficiencies on the normal blood picture.

## EXPERIMENTAL

The animals used in these studies were young (1½ to 2 years) rhesus monkeys of both sexes that were secured directly from an animal dealer. The monkeys were kept on a general diet of bread, lettuce, carrots, cereal, and fruit for about 5 days, during which time they were given a tuberculin test. Following this period the

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TABLE 1.—A Summary of the Blood Picture of Rhesus Monkeys Reported by Various Workers

Reference	Number of Monkeys	Hemoglobin <i>Gm. per 100 ml. blood</i>	Erythrocyte Count <i>Millions per cu. mm.</i>	Total Leukocyte Count <i>Thousands per cu. mm.</i>	Differential Leukocyte Count					
					Monocyte %	Neutrophil %	Lymphocyte %	Eosino- phil %	Mast %	Baso- phil %
Anderson and Neill <sup>13</sup> 1915	10		4.64(20) <sup>a</sup>	11.1(100)		41.6	53.6 <sup>b</sup>	3.7	0.3	
Sellards and Baetjer <sup>6</sup> 1915	1			25.0		40	60 <sup>b</sup>			
Wells and Sutton <sup>7</sup> 1915	0		6.21	5.2						
Taylor <sup>8</sup> 1919	40			22.1(121)	0.6	41.1	53.7	4.1		0.4
Krumhaar and Musser <sup>9</sup> 1921	14		5.98	13.3	4.9	47.4	46.5	1		0.2
Krumhaar and Musser <sup>10</sup> 1923				14.2			gross small			
Klieneberger <sup>11</sup> 1927			6.35	7.5	1.3	35.8	12.46	3.5	0.3	
Fox <sup>12</sup> 1927	8		4.50-7.00	10.0 13.0	5.6	44-51	33-47	0-11		
Ponder et al. <sup>13</sup> 1929	8		5.00	10.4	2.0	73	21	3.0		1.0
Hall <sup>14</sup> 1929			4.94	14.1	1.2	42.6	51.8	2.8	0.3	
Harmon et al. <sup>15</sup> 1931			6.20(434)	19.5(272)	1.9	47.9	46.7	2.9		0.4
Bilimoria <sup>16</sup> 1931	27 <sup>d</sup>	12.53	6.43	18.1	2.0	35	61	2.0		
Wills and Stewart <sup>17</sup> 1935	13 <sup>e</sup>	12.2(107)	6.20(113)	15.2(116)	2.4	41.5	52	2.1		0
Verder and Petrant <sup>18</sup> 1937	3		4.54-5.37 (9-14)	11.0-17.8 (9-14)	0.7-0.9	30.4-43.5	54.1-67.4	0-1.7		
Shukers et al. <sup>1</sup> 1938	19 <sup>f</sup>	12.2(140)	5.20(143)	15.1(149)	3.0	36.0	59.0	0.5		0.5
Rao et al. <sup>19</sup> 1940	13 <sup>g</sup>	14.1	5.69-6.30	16.6	2.7	31.9	62	3.2	0.2	
Suarez et al. <sup>2</sup> 1942	8 infant 4 young 26 adult	12.2(1) 13.1(1) 13.2(25)	4.19(1) 5.17(1) 5.23(1)	15.1(1) 17.5(1) 9.9(25)		36.1 38 50.2	57.7 56.7 42.2	3.06 4 4.9		0.44 0
Cooperman et al. <sup>20</sup> 1945	10 young	14.0-15.5(50)	4.0-5.5(50)	10-25 α(50)	0.75					

<sup>a</sup> Numbers in parentheses indicate number of observations.<sup>b</sup> Includes monocytes.<sup>c</sup> *Caecopithecus callitrichus*.<sup>d</sup> Adult *M. rhesus* and *M. sinensis*.<sup>e</sup> Immature—just after 2nd stage of dentition.<sup>f</sup> Young rhesus monkeys.<sup>g</sup> *M. sinensis*.

animals were fed the M-3 basal diet supplemented with various liver products. The basal diet consisted of the following parts per 100: sucrose 73, vitamin-free casein 18, salts IV 4, cod-liver oil 3, and corn oil 2. In addition, each animal received the following vitamin supplement per day: thiamine hydrochloride 0.5 mg., riboflavin 0.5 mg., pyridoxine hydrochloride 1.0 mg., calcium pantothenate 3.0 mg., niacin 5.0 mg., choline chloride 25 mg., i-inositol 50 mg., p-aminobenzoic acid 50 mg., biotin 20 ug., folic acid 100 ug., and vitamin C 25 mg.

The ration designated as M-5 was similar to the M-3 ration but contained 3 per cent bovine globulin and 2 per cent yeast added at the expense of the sucrose. This ration was also supplemented with 3 per cent whole liver substance and the animals

TABLE 2.—*Blood Studies on Monkeys Maintained on Practical Natural Diets*

	Monkey Number	Hemo- globin	Erythro- cyte Count	Total Leuko- cyte Count	Differential Leukocyte Count					
					Mono- cyte	Neutro- phil	Lym- phocyte	Eosino- phil	Mast Cell	Unclassi- fied
		Gm. per 100 ml.	Millions per cu. mm.	Thous- ands per cu. mm.	%	%	%	%	%	%
Monkeys recently acquired from dealers	306	12.48	6.31	13.1	3.5	35.0	54.0	7.0	0.5	0
	307	12.84	6.03	10.9	2.5	35.5	57.5	3.5	1.0	0
	308	12.21	6.86	17.9	3.5	29.0	59.5	7.0	1.0	0
From zoo	210	13.58	6.78	14.2	2.5	78.0	19.5	0	0	0
	211	12.84	6.01	20.8	3.0	58.0	34.0	4.5	0	0
	212	11.21	5.09	16.7	1.0	41.5	56.0	1.5	0	0
	213	14.16	6.55	10.6	3.0	58.5	34.5	3.5	0	0
	214	14.55	6.38	15.6	5.0	32.5	59.0	2.5	0	0
	215	13.38	7.33	13.3	0.5	70.5	25.0	2.0	0	0
	216	12.33	5.11	16.3	1.5	34.0	62.0	2.0	0	0
	284	13.11	6.51	15.6	2.0	43.0	53.5	1.5	0	0
	285	13.77	6.74	12.4	2.5	46.0	50.0	1.0	0	0.5
	286	11.98	6.49	26.4	4.5	46.0	47.5	2.0	0	0
	287	12.49	7.15	16.2	2.5	47.0	46.5	3.5	0.5	0
	288	12.06	6.38	25.8	2.5	38.5	56.5	2.0	0.5	0
Ave.....		12.95	6.38	16.9	2.5	49.5	45.3	2.2	0.1	0

receiving it were given a double quantity of the vitamin supplement. The M-6 ration was prepared by replacing 40 per cent of the M-3 ration with ground corn grits. This ration was supplemented with 3 per cent whole liver substance, the regular vitamin supplement, and 50 cc. raw milk per day.

Each monkey was bled in the morning, usually before feeding, from the marginal veins of the ear, or in later experiments from the saphenous vein of the leg since this gave a more adequate blood sample from young monkeys. Hemoglobin was determined with the Evelyn photoelectric colorimeter. The total leukocyte and erythrocyte counts were made in the usual way. Dry smears, stained with Wright's stain, were used for the differential counts and the neutrophil and lymphocyte counts were expressed as per cent of the total white cell count. One hundred

TABLE 3.—*The Composition of Blood from Monkeys Fed Different Complete Diets*

Diet	Monkey Number	Hemoglobin	Erythrocyte Count	Total Leukocyte Count	Differential Leukocyte Count					
					Monocyte	Neutrophil	Lymphocyte	Eosinophil	Mast Cell	Unclassified
		Gm. per 100 ml.	Millions per cu. mm.	Thousands per cu. mm.	%	%	%	%	%	%
M-3 + 3% whole liver substance	1	15.91	5.60	15.8	13.0	48.5	35.5	2.5	0.5	0
	1	14.28	6.11	18.2	4.5	24.0	60.5	10.5	0.5	0
	139	14.16	6.67	16.0	2.5	42.0	54.0	1.0	0.5	0
	261	15.91	5.71	12.3	5.5	19.5	74.5	0.5	0	0
	261	14.86	6.11	13.5	5.0	22.5	65.0	6.5	0.5	0.5
	286	15.17	6.46	24.0	3.0	28.0	62.5	6.5	0	0
	286	15.05	6.52	28.4	4.5	22.0	67.5	6.0	0	0
	287	14.78	5.87	13.8	6.5	37.0	54.0	2.5	0	0
	287	14.47	6.99	18.3	2.5	42.0	53.0	2.0	0.5	0
	300	14.35	7.19	27.9	1.0	36.0	58.0	4.5	0	0.5
	300	14.16	6.90	18.7	3.5	24.0	70.0	2.5	0	0
	301	14.35	6.35	10.3	3.0	28.0	63.0	5.5	0.5	0
Ave. ....		14.87	6.37	18.1	4.5	31.1	59.8	4.2	0.25	0.10
M-3 + 3% liver extract	116	13.89	6.17	11.7	2.5	19.5	66.5	11.5	0	0
	200	14.08	6.87	22.7	1.0	34.0	62.0	2.75	0	0.25
	208	13.50	5.76	10.0	7.0	33.0	55.0	3.5	0	1.5
	209	13.03	6.48	17.8	6.0	27.5	52.5	13.5	0.5	0
	284	14.35	6.68	16.1	1.5	38.0	58.0	2.0	0.5	0
	285	14.16	6.61	15.8	3.5	43.0	51.5	2.0	0	0
	298	13.58	5.26	18.0	1.0	55.0	40.0	4.0	0	0
	299	13.30	5.20	17.0	1.0	29.0	69.0	1.0	0	0
	314	14.16	7.04	16.2	2.0	59.5	34.5	2.5	1.5	0
	315	15.91	7.16	22.6	2.5	25.0	67.0	3.5	1.0	1.0
	316	15.17	6.71	9.7	3.0	22.0	74.0	0	1.0	0
	317	14.28	6.76	22.2	3.5	44.5	48.5	1.5	1.0	1.0
Ave. ....		14.12	6.39	16.6	2.9	35.9	56.5	3.9	0.4	0.3
M-5 + 3% whole liver substance	267	13.30	6.53	27.0	5.5	33.5	55.5	4.0	0	1.5
	268	13.23	7.47	21.2	1.0	45.5	52.0	1.5	0	0
	269	14.35	7.64	29.2	2.5	19.0	72.0	6.5	0	0
	269	14.78	6.90	16.1	2.0	14.0	80.0	4.0	0	0
	272	15.48	6.41	17.6	2.5	38.5	55.5	2.5	0	1
	273	14.08	6.46	22.3	5.0	22.5	72.0	0.0	0.5	0
	288	14.86	5.65	19.6	4.0	19.5	72.0	4.0	0.5	0
	288	14.55	6.08	36.3	2.0	24.0	68.0	6.0	0	0
	302	17.04	6.38	20.0	5.0	20.5	68.0	6.5	0	0
	302	14.78	6.56	21.0	1.5	24.0	67.5	7.0	0	0
	304	14.86	7.36	12.9	3.5	36.0	51.0	7.5	2.0	0
	304	14.55	6.55	13.4	8.0	23.5	59.0	8.0	1.0	0.5
Ave. ....		14.65	6.66	21.4	3.5	26.7	64.4	4.8	0.3	0.2
M-6 + 3% whole liver substance	310	14.28	5.40	18.6	6.0	20.0	59.5	11.5	1.0	2.0
	311	13.50	5.54	28.4	4.0	24.5	68.0	3.5	0	0
	311	15.05	5.75	26.7	4.5	22.5	66.5	4.5	2.0	0
	312	13.50	6.36	18.3	2.5	30.5	63.5	1.5	0.5	1.5
	312	13.78	6.15	23.2	2.0	42.5	49.5	5.5	0.5	0
	312	14.16	5.96	20.2	3.0	32.0	62.5	2.0	0.5	0
	313	12.33	5.78	18.1	2.5	18.5	68.0	10.5	0	0.5
	313	13.89	6.71	16.8	2.7	27.0	58.5	12.0	0	0
	313	13.89	5.88	15.4	3.0	26.5	58.0	12.0	0.5	0
Ave. ....		13.82	5.95	20.6	3.3	27.1	61.5	7.0	0.5	0.4

white cells were counted in each of two different areas of the smear. If a discrepancy was found between these two counts 200 or 300 more cells were counted. Because of the recognized difficulty of distinguishing the monocytes from the large lymphocytes, we included among the monocytes those cells showing a comparatively smooth homogenous nuclear structure, which stained more lightly and which was buckled or folded, usually with fine azurophilic dust in the cytoplasm. Otherwise, the large cells were classified as lymphocytes.

Table 2 contains the blood records of 3 young monkeys recently acquired from the dealer and maintained for 4 weeks on the general practical ration as described above. This table also includes the data for 12 monkeys that were kept at a local zoo for 3 months and which had access to the following types of food: fortified white bread, cabbage, carrots, swiss chard, bananas, oranges or apples. The blood picture of these two groups of animals was similar and the values agree in most instances with the data of Shukers et al.<sup>1</sup> Although the erythrocyte count was slightly higher than most of the previously reported values, the per cent of neutrophils in the blood of the monkeys from the zoo tended to be higher than in those from the dealer.

All monkeys receiving supplements of whole liver substance showed higher hemoglobin concentrations, similar white blood cell counts, but a considerably lower neutrophil and higher lymphocyte differential count than those animals fed natural diets. The blood picture of representative monkeys fed supplements of whole liver substance is presented in table 3.

When 3 per cent liver extract was fed as the liver supplement to the M-3 ration, the animals showed about the same blood values as those fed whole liver substance except that the neutrophil-lymphocyte ratio tended to rise from a normal value of

$0.5 \left( \frac{30}{60} \right)$  to values ranging up to  $2.0 \left( \frac{60}{30} \right)$ .

#### BLOOD PICTURE IN VITAMIN DEFICIENCIES

It is of interest to note the various characteristic blood dyscrasias that result in the rhesus monkey from a deficiency of certain members of the vitamin B complex. Deficiencies of pyridoxine,<sup>22</sup> riboflavin,<sup>20</sup> pantothenic acid,<sup>22</sup> folic acid,<sup>23, 24</sup> and the monkey antianemia factor<sup>23, 24</sup> each result in an anemia. Folic acid deficiency is also characterized by a severe leukopenia. Chronic deficiencies of pyridoxine, riboflavin, pantothenic acid or folic acid show in addition an abnormally high neutrophil-lymphocyte ratio. It has been shown recently<sup>20, 22, 24</sup> that this effect is due to a concomitant deficiency of the monkey antianemia factor. Supplies of whole liver are required in addition to pyridoxine, riboflavin, pantothenic acid or folic acid for complete blood regeneration. All of these changes are summarized in figure 1. Typical changes in the differential count of the blood from a single monkey developing a deficiency of the monkey antianemia factor are shown in figure 2.

Two monkeys fed diets containing egg white and supplied with excess biotin (100 ug. per day) have shown consistently high leukocyte counts. One of these showed a neutrophil-lymphocyte ratio of 2.0 (table 4).

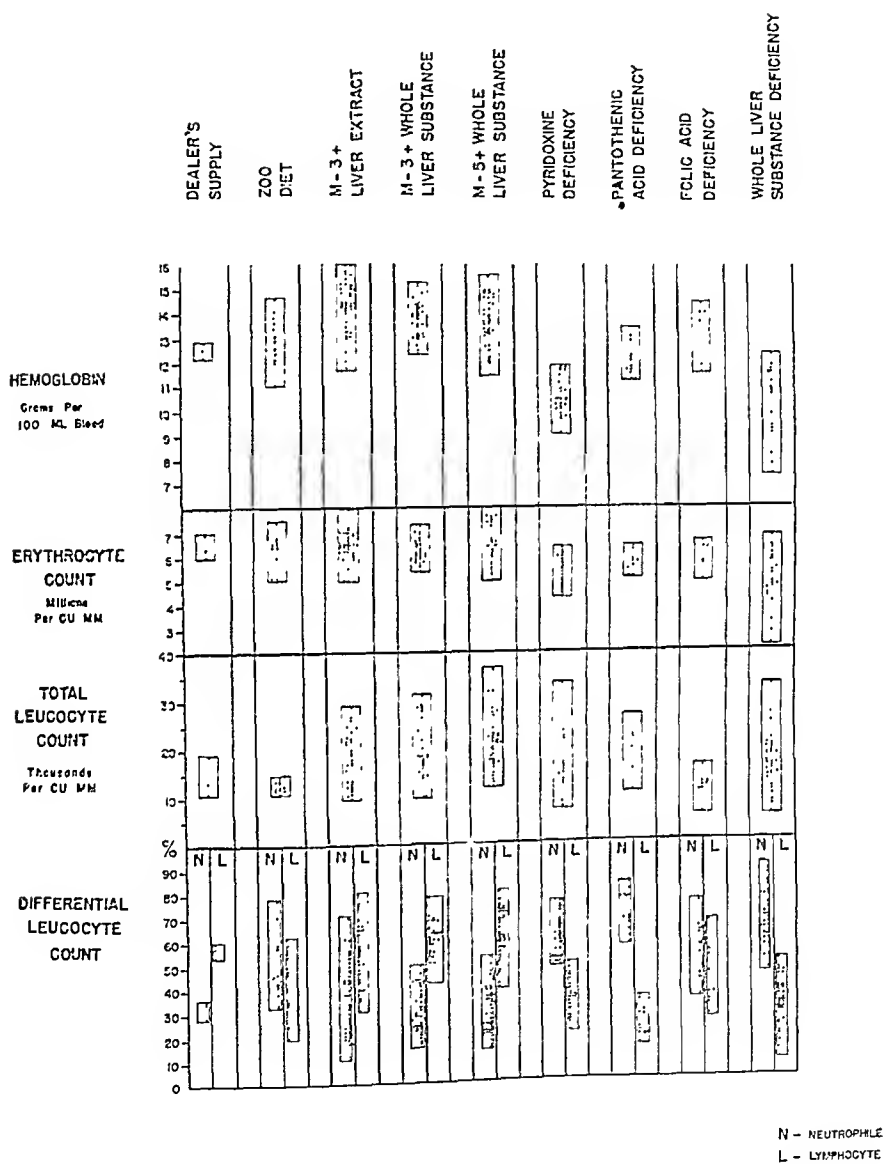


FIG. 1. HEMOGRAM OF THE RHESUS MONKEY UNDER CERTAIN DIETARY CONDITIONS

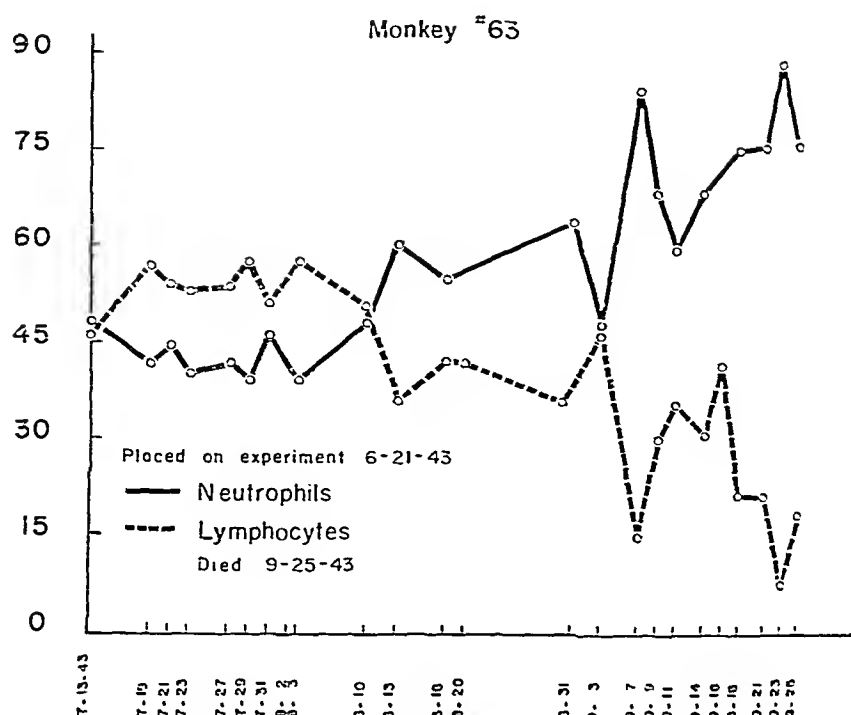


FIG. 2. CHANGE IN PERCENTAGE OF NEUTROPHILS AND LYMPHOCYTES WITH THE PROGRESS OF WHOLE LIVER FACTOR DEFICIENCY

TABLE 4.—Blood Studies on Monkeys Fed Egg White Rations with Excess Biotin

Monkey Number	Time on Diet	Hemo- globin	Erythrocyte Count	Total Leukocyte Count	Differential Leukocyte Count					
					Mono- cyte	Neutro- phil	Lym- phocyte	Eosino- phil	Mast Cell	Uniden- tified
	Days	Gm. per 100 ml.	Millions per cu. mm.	Thousands per cu. mm.	%	%	%	%	%	%
253	96	13.03	7.57	33.1	5.0	56.5	37.0	1.0	0.5	0
253	108	14.28	7.14	37.5	3.5	57.5	35.0	3.5	0.5	0
253	116	13.50	7.95	30.4	7.0	56.0	33.5	3.5	0	0
253	124	—	—	30.7	—	—	—	—	—	—
253	129	14.78	8.39	42.2	3.5	68.0	28.0	0.5	0	0
278	41	12.91	6.65	23.8	4.0	35.5	52.0	8.0	0	0.5

#### DISCUSSION

The data in figure 1 indicate that wide variations in the total leukocyte count of the blood of monkeys on different diets are frequently seen. Although the variation in the white count is not completely explained, it is quite probable that many high white cell counts are the results of excitement of the animals unaccustomed to being subjected to blood tests. It has been observed in our laboratory that the first blood examination of many newly acquired monkeys is characterized by a high

white count. After the animal has been handled several times the leukocyte counts not only tend to be lower than the initial counts but also are more uniform, unless subsequently altered by the dietary regimen. In the case of folic acid deficiency, a severe leukopenia develops but the record of the total white cell count occasionally shows an abnormally high value. These increases are probably the result of a response to the intercurrent infections such as dysentery to which folic acid-deficient monkeys are particularly susceptible.<sup>23</sup>

A cell common to the monkey has been described by Fox<sup>12</sup> as a basophilic neutrophil and by Hall<sup>14</sup> as the mast cell. Our data indicate that a cell which seems to be identical with the mast cell of Hall is present frequently (0-1 per cent). Suarez et al.<sup>2</sup> have described the presence of metamyelocytes in the peripheral blood of rhesus monkeys of all ages but we have not observed this cell.

As a result of recent advances in the knowledge of the nutritional requirements of the rhesus monkey it has become possible to obtain a more exact picture of the concentration of hemoglobin and the formed elements in the blood of the normal monkey. Using the purified rations as described and supplemented with all the crystalline B vitamins and one of several liver products, the blood picture has been found to be considerably higher than that previously accepted as the normal picture. When whole liver products are fed as supplements to the purified rations, the hemoglobin and erythrocyte counts are higher, and the neutrophil-lymphocyte ratio is lower than when liver extracts are given as supplements or when certain mixed diets are used. One monkey fed the M-3 basal diet supplemented with 3 per cent liver extract for four years ultimately developed a reversed neutrophil-lymphocyte ratio, a mild anemia, and failed to maintain its body weight. Three weeks after substituting whole liver substance for the liver extract, the blood picture of this animal was completely restored to the high levels found in those animals fed purified rations supplemented with whole liver substance from the beginning.

Thus the great variation in the differential count reported by many of the previous workers may be accounted for in part by the use of an inadequate diet. For

example, we have shown that a high neutrophil-lymphocyte ratio  $\left(\text{e.g., } \frac{60}{30}\right)$  ultimately results from single deficiencies of four different B vitamins and that when

these rations are adequately supplemented with all the vitamins and a supply of

whole liver substance, the neutrophil-lymphocyte ratio is about  $0.5 \left(\frac{30}{60}\right)$ . It should

be noted that Suarez et al.<sup>2</sup> have reported a high neutrophil-lymphocyte ratio  $\left(\frac{50}{42}\right)$

in adult monkeys in contrast to the low neutrophil-lymphocyte ratio  $\left(\frac{36}{57}\right)$  of in-

fant monkeys. During the first few months of postnatal life the major component of the diet of the monkey is milk supplied by the mother and we have found that

this supports excellent growth and a normal blood picture in the infant rhesus monkey.<sup>25</sup>

The blood dyscrasias which we have described that result from single deficiencies of pyridoxine, pantothenic acid, riboflavin, folic acid, and the monkey anti-anemic factor have been found to be characteristic of each deficiency. These alterations, such as the acute leukopenia of folic acid deficiency, generally precede and are ascertainable before outward deficiency signs develop to any significant extent. Hence these blood examinations are of special value when used in connection with growth data and outward deficiency signs to determine accurately the state of deficiency that exists in an individual monkey.

#### SUMMARY

1. Rhesus monkeys fed purified rations supplemented with adequate amounts of the B vitamins, ascorbic acid, and whole liver substance maintained the following average blood picture:

Hemoglobin . . . . .	14.5 Gm.%
Red blood cells . . . . .	6.33 millions per cu. mm.
Total leukocyte count . . . . .	20.0 thousands per cu. mm.
Differential leukocyte count	
Neutrophils . . . . .	28.3%
Lymphocytes . . . . .	61.9%
Monocytes . . . . .	3.8%
Mast cells . . . . .	0.3%
Eosinophils . . . . .	5.3%

2. Natural diets or purified rations supplemented with liver extract do not support the above blood picture. The hemoglobin is lower and there is an increase in the range of the total leukocyte count and in the neutrophil-lymphocyte ratio

to  $2.0 \left( \frac{60}{30} \right)$ . These figures are similar to the values in the literature and generally accepted as the normal.

3. Previous reports have shown the characteristic blood dyscrasias which develop when monkeys are fed certain B vitamin-deficient diets. These changes are summarized graphically in this paper.

4. The importance of determining the concentration of hemoglobin and the formed elements of the blood as a diagnostic test in nutritional studies has been shown.

We wish to acknowledge our indebtedness to Merck and Co., Rahway, N. J., for some of the crystalline vitamins; to Wilson Laboratories, Chicago, Ill., for the various liver preparations; and to Lederle Laboratories, Inc., Pearl River, N. Y., for synthetic folic acid.

The authors are grateful to Miss Ethel Thewlis for aid in determining cellular elements and to Drs. Harry A. Waisman and James H. Shaw for assisting in early parts of the work.

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## HEMOPOIESIS IN RIBOFLAVIN-DEFICIENT RATS

By K. M. ENDICOTT, M.D., ARTHUR KORNBERG, M.D., AND  
MAURINE OTT, A.B.

IN THIS laboratory, experimental granulocytopenia and anemia in rats have been produced by a number of dietary deficiencies, including "folic acid" (pteroyl-glutamic acid) deficiency,<sup>1, 2</sup> riboflavin deficiency,<sup>3</sup> pantothenic acid deficiency,<sup>4</sup> and deficiency of essential amino acids.<sup>5</sup> A detailed study of the hemopoietic tissues in folic acid deficiency and their response to folic acid therapy has been reported.<sup>6</sup> The present study is a comparison of hemopoietic tissues in the granulocytopenia and anemia of riboflavin deficiency with the hemopoietic tissues in folic acid deficiency, including the response of these tissues to vitamin therapy.

In the studies of Kornberg, Daft, and Sebrell,<sup>3</sup> rats fed a diet deficient in riboflavin developed granulocytopenia in about 50 per cent of the cases and, less frequently, anemia. The granulocytopenia responded to treatment with folic acid in 30 or 33 rats but responded to riboflavin therapy in only 6 of 28 rats. Riboflavin alleviated the anemia in 10 of 17 rats while folic acid failed to do so in all of 7 rats. In the present study, therefore, rats having granulocytopenia were treated with folic acid while rats having both granulocytopenia and anemia were treated with riboflavin.

The experimental conditions with few exceptions have been similar to those of the previous study of hemopoiesis in folic acid deficiency<sup>6</sup> so as to permit accurate comparison of hemopoiesis in the two deficiencies. Both studies were carried out by a quantitative method in which an index of the total quantity of each type of cell in the bone marrow is obtained. This is accomplished by determining the average cellularity of the marrow and the differential count of marrow cells. The average per cent of cellularity is multiplied by the per cent in the differential to arrive at a quantitative index of the total amount of each type of marrow cell.

### EXPERIMENTAL METHODS

At weaning, albino rats of both sexes of Osborne and Mendel strain were fed purified diet number 950<sup>3</sup> ad libitum. This consisted of leached, alcohol-extracted casein (18 per cent), dextrose (68.76 per cent), hydrogenated vegetable oil (8 per cent), salt mixture (4 per cent),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.08 per cent), and ferric citrate (1.16 per cent). To each 100 grams of this diet were added thiamine hydrochloride (1 mg.), pyridoxine hydrochloride (1 mg.), calcium pantothenate (4 mg.), nicotinamide (2 mg.), 2-methyl-1,4-naphthoquinone (vitamin K) diacetate (0.4 mg.), biotin (0.001 mg.), and choline chloride (200 mg.) dissolved in 1.0 cc. of 20 per cent ethanol. Twice weekly each rat received 0.25 cc. of corn oil containing 2000 units of vitamin A and 200 units of vitamin D, and once weekly 3 mg. of  $\alpha$ -tocopherol in 0.03 cc. of ethyl laurate. The study extended over a period of 15 months.

From the Pathology Laboratory (K. M. Endicott and Maurine Ott) and the Division of Physiology (Arthur Kornberg), National Institute of Health.

Eight rats from two or more litters were placed on the experimental regimen each week. Within the first week the following determinations were carried out on each rat using freely flowing tail blood: hemoglobin (Evelyn photoelectric colorimeter), hematocrit (van Allen), erythrocyte count (in duplicate, diluting in Trenner pipets), leukocyte count (in duplicate, Trenner pipets), differential leukocyte count (200 cells), and reticulocyte count (with the Miller disk\*).

The rats were observed daily and the leukocyte and differential counts and the hematocrit determinations were repeated when any of the following signs were noted: facial porphyrin stains, pallor, loss of hair, dermatitis, general weakness, and loss of weight. When these check counts indicated that the rats were suitable for special hematologic study, complete blood determinations, as above, were repeated and the rat was studied under one of the following experiments:

(a) In order to study the hemopoietic tissues of rats with severe granulocytopenia, 12 rats were killed when their polymorphonuclear counts fell below 150 per cubic millimeter.

(b) A corresponding group of 21 rats with similar or more severe granulocytopenia were given daily oral doses of 25 micrograms of crystalline folic acid† for periods of 4 days (8 rats), 8 days (6 rats), and 12 days (7 rats). In each case complete blood determinations were carried out at the end of the treatment period and the rats were killed for detailed studies of hemopoietic tissues.

(c) Another series of 11 rats with both granulocytopenia and anemia (hematocrit below 30 vol. per cent) were studied without treatment for comparison with the series (a) rats showing granulocytopenia alone.

(d) A group of 4 rats with hematocrit values below 30 vol. per cent were treated for 10 days with daily oral doses of 100 micrograms of riboflavin and were killed and examined for the purpose of noting the effect of this therapy on the hemopoietic tissues.

(e) For the purpose of studying the evolution of the dyscrasias rats were selected according to the following criteria: (1) The blood picture must have been normal at the beginning of the experiment. (2) A period of at least 1 month on diet number 990 must have elapsed. (3) A steady downward trend of the granulocyte count must have been shown. (4) There must have been no anemia at any time. Thus this series comprised 7 rats with polymorphonuclear counts over 1000 per cubic millimeter, 4 rats with counts between 500 and 1000, 5 rats with counts between 150 and 500, and 12 rats with counts of less than 150 polymorphonuclears per cubic millimeter.

In all experiments the blood of each rat was examined on the day of death. Rats were killed with illuminating (mixed natural and coal) gas. Giemsa-stained smears of marrow from the left femur were prepared by the plasma diluting method,<sup>7</sup>

\* Microscope ocular disk designed by Dr. John W. Miller, manufactured by Bausch and Lomb Optical Company. The disk is ruled with one square ( $7 \times 7$  mm.) containing a smaller square in one corner having one ninth the area of the larger square. For each of 50 fields the erythrocytes in the smaller square and the reticulocytes in the larger square are counted.  $\text{Reticulocytes} \div 9 \times \text{erythrocytes} = \% \text{ reticulocytes}$ .

† Furnished by courtesy of Dr. E. L. R. Stokstad and B. L. Hurchings, Lederle Laboratories, Inc.

# ABSTRACTS

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## ERYTHROCYTES AND ERYTHROCYTIC DISEASE

THE TREATMENT OF MACROCYTIC ANEMIA WITH LACTOBACILLUS CASEI FACTOR (PTEROYLGLUTAMIC ACID).  
*G. A. Goldsmith. J. Lab. & Clin. Med. 31: 1186-1200, 1946.*

Careful clinical and hematologic studies were made on a series of 15 patients with various types of anemia who were treated with *L. casei* factor (pteroylglutamic acid, folic acid).

Three cases of pernicious anemia showed clinical and hematologic improvement following oral or parenteral treatment with *L. casei* factor. Improvement was of a degree comparable to that which would have been observed following liver extract therapy, although the reticulocyte responses were not as great following *L. casei* factor therapy.

One patient with definite signs of subacute combined degeneration of the spinal cord showed marked improvement following *L. casei* factor therapy. Complete clinical and hematologic remission was maintained for a period of six months in 1 patient on 15 mg. of *L. casei* factor daily. Four patients with nutritional macrocytic anemia responded satisfactorily to *L. casei* factor treatment. Two cases of sprue with macrocytic anemia improved clinically with decrease in fatty diarrhea and gain in weight when given *L. casei* factor, but showed no significant improvement in anemia.

One case of celiac disease showed improvement in clinical condition and slight improvement in anemia. No improvement followed *L. casei* factor therapy in 2 patients with aplastic anemia, 1 case of unexplained macrocytic anemia with hyperplastic bone marrow, 1 case of myxedema with macrocytic anemia, and 1 case of regional ileitis with anemia.

J. F. R.

AN UNIDENTIFIED FACTOR OR FACTORS EFFECTIVE IN THE TREATMENT OF EXPERIMENTAL BLOOD DYSCRASIAS IN RATS. *F. S. Daft and W. H. Sebrell. Fed. Proc. 5: 231, 1946.*

It has been shown that rats deprived of pantothenic acid develop anemia and granulocytopenia, and that pantothenic acid prevents the production of these abnormalities. In cases in which the granulocytopenia developed without concurrent anemia, *L. casei* factor (folic acid) was able to correct the neutropenia without the use of pantothenic acid.

Daft and Sebrell gave rats a pantothenic-acid-deficient diet identical with the one used in the above experiments, except that it contained folic acid in amounts of 4 micrograms per gram of food. Rats on this diet developed both anemia and granulocytopenia. When this condition occurred, treatment with whole dried liver was more effective than treatment with pantothenic acid alone. It was therefore concluded that there is probably in liver an additional factor, neither folic acid nor pantothenic acid, which prevents anemia and granulocytopenia due to dietary deficiency.

S. E.

ADDISONIAN ANEMIA FOLLOWING ENTERO-ANASTOMOSIS. *J. E. Richardson. Brit. J. Surg. 33: 71-74, 1946.*

According to this report, there are some 51 cases in the literature of macrocytic anemia in association with intestinal strictures and anastomoses. Richardson finds that these cases can be explained by the postulate that pernicious anemia probably results from the lack of a hemopoietic principle whose function it is to maintain the integrity of the intestinal mucosa. Lack of this factor, he states, allows the absorption of "toxins" which result in the various changes of pernicious anemia. Other causes of damage to the

intestinal mucosa may result in the same picture. Thus, in intestinal stagnation there is excessive formation of toxins which may in themselves cause damage to the mucosa even in the presence of normal amounts of the hemopoietic principle. In ileocolic anastomosis, the normal hemopoietic principle is not absorbed, and in addition the stagnant loop below the site of anastomosis may produce mucosa-damaging "toxins."

Richardson's case report concerns a 20 year old man who required jejunoileic anastomosis because of intestinal obstruction following an acute appendiceal abscess. Pain, diarrhea, and vomiting occurred occasionally during the following five years. Examination at the end of this time revealed increased gastric acidity and a duodenal ulcer, and a macrocytic hyperchromic anemia with blood smears typical of pernicious anemia. No bone marrow examination was done. Treatment with liver extract caused moderate reticulocytosis. Subsequent reoperation with elimination of the jejunoileostomy and restoration of the normal continuity of the bowel was done, following which the blood counts returned to normal and the complaints disappeared. Temporary postoperative treatments with blood transfusion, liver extract, vitamins, and iron were stopped, and the patient seemed cured.

The usual explanation for the development of macrocytic anemia after gastrectomy, gastroenterostomy, etc., is the elimination of the intrinsic factor or the hampering of its absorption. It is interesting that, of the 51 cases mentioned in the literature, 18 were associated with various enteroanastomoses, so that the possible role of mucosal damage and "toxins" postulated by Richardson may have to be seriously considered in further attempts to determine the etiology of this condition.

S. E.

ACUTE HEMOLYTIC ANEMIA ASSOCIATED WITH AUTOAGGLUTINATION WITH A THERMAL AMPLITUDE OF 0 TO 37° C. *H. Lubinski and A. Goldbloom. Am. J. Dis. Child. 72: 325-333, 1946.*

A fatal case of acute hemolytic anemia in a boy aged 11 years is described. The patient's serum contained autohemagglutinins active against autologous and other group A red cells and group O cells in a titer of 1:4 at refrigerator, room, and body temperatures. The agglutinins were completely absorbed with A and O cells at all three temperatures. "Purified agglutinins," absorbed by group A cells at both refrigerator and body temperatures and washed into saline at 56° C., were active against group A and group O human cells, and also against erythrocytes of rabbit, duckling, and sheep. To the best of the reviewer's knowledge, this is the seventh reported case of hemolytic anemia associated with autohemagglutinins active at body temperature. Five of the 7 cases have terminated fatally. In 3 of the 7 cases, including the case described in this report, positive Wassermann reactions were observed.

An excellent discussion of the possible origin and significance of autoagglutinins is presented. Lubinski and Goldbloom suggest that the antigen for autoagglutinins may be composed of (1) a substance derived from toxic or infectious agents and (2) some part of the red cells, and that the combination may alter the erythrocyte so that it acts as an antigen in and against its own serum. The occurrence of autoagglutinins in nonhemolytic diseases is emphasized and the authors express the opinion that autoagglutinins are never primarily responsible for hemolysis *in vivo*. No mention is made, however, of the fact that increased mechanical fragility of agglutinated erythrocytes has been demonstrated by other investigators. It is reasonably concluded that the exact role of these peculiar antibodies in hemolytic anemia is far from clear.

L. E. Y.

ERYTHROCYTE FRAGILITY IN ACUTE INFECTIOUS HEPATITIS. *D. F. Bellet. J. Clin. & Lab. Med. 31: 1179-1185, 1946.*

Icteric indices and the susceptibility of the erythrocytes to hemolysis in hypotonic salt solutions ("erythrocyte fragility") were determined in 47 soldiers with acute infectious hepatitis during brief periods of observation. Definite decrease in erythrocyte fragility (increased resistance) was observed during the icteric phase of the disease, but this decreased erythrocyte fragility was not a function of the bile pigment concentration of the plasma.

One *in vitro* experiment is reported in which normal erythrocytes were incubated with icteric serum and erythrocytes from a jaundiced patient (with increased fragility) were incubated in normal serum. Neither type of cell showed any change in fragility.

The author proposes the hypothesis that the observed fragility changes are not due to the effect of some abnormal plasma constituent acting directly on the erythrocytes, but reflect a more fundamental disturbance in erythropoiesis produced as a result of diffuse liver damage.

J. F. R.

CHRONIC HEMOLYTIC ICTERUS RESEMBLING ACHOLURIC JAUNDICE OCCURRING IN AN AFRICAN NATIVE  
*C. Merskey and E. Baskind*. S. African Med. J. 20: 230-233, 1946.

A 25 year old Msutu male from Northern Transvaal was studied because of persistent pain in both legs. He was found to have old healed scars on the legs, tenderness to pressure over both tibiae, mottled pigmentation of the tongue, and a spleen palpable two fingers' breadth below the costal margin. The icterus index was never higher than 18. Spherocytosis and increased osmotic fragility of the red cells were demonstrated repeatedly. The red cell count fell only to 3,290,000 during an acute febrile period at which time both liver and spleen became larger. Bone marrow puncture revealed marked erythroid hyperplasia, but x-rays of the tibiae and skull were normal. Although hemolysins could not be demonstrated, cold hemagglutinins were present in low titer. Splenectomy was refused by the patient. Other members of the family could not be examined; both parents had died of unknown cause.

Merskey and Baskind state that this is the first case of chronic hemolytic icterus to be reported in an African native. Wintrobe (Textbook of Clinical Hematology) has, however, encountered congenital hemolytic jaundice in a Negress of mixed blood, and Scherer and Cecil (J. Lab. & Clin. Med. 30: 244-246, 1945) have reported another case in a Negress in whose family miscegenation could not be excluded. Although this case conforms to the usual pattern of congenital hemolytic icterus, the authors prefer to leave open the question as to whether the disease in this patient was congenital or acquired. The possible role of malignant tertian malaria is also considered since the patient lived in a malarious area and rings were found in a blood smear on one occasion. The conservatism of the authors with regard to exact diagnosis is probably justified in view of current uncertainties in the classification of hemolytic disorders.

L. E. Y.

HEMOLYTIC DISEASE OF THE NEWBORN (ERYTHROBLASTOSIS FETALIS): TREATMENT BY A SINGLE MASSIVE TRANSFUSION WITH COMPLETE RECOVERY. *A. Bloxsum*. Am. J. Dis. Child. 72: 320-324, 1946.

A group A, Rh positive infant with erythroblastosis fetalis (mother group O, Rh negative, father group A, Rh positive) accidentally received a transfusion of 400 cc. of group A, Rh negative blood. The infant's condition remained satisfactory following transfusion; icterus of the skin was not noted.

Whereas erythrocytes of the cord blood had reacted weakly with anti-Rh serum, the infant's red cells 9 days after the massive transfusion reacted strongly. The author therefore postulates that the infant received an "anti-Rh antigen-antibody reaction factor" in the transfused blood which permitted his own Rh positive cells to survive. Bloxsum is of the opinion that Rh negative persons may have in their blood a suppressing (or anti-Rh antigen-antibody reaction) factor which "cushions" the antigen-antibody reaction. He therefore suggests that use be made of this inhibitory substance by giving to erythroblastic infants larger transfusions of Rh negative blood than are customary (although not as large as given accidentally in this case).

There is some evidence in the literature that substances capable of inhibiting hemolysis and hemagglutination are present in both normal and pathologic human sera. It is unfortunate, however, that the speculations advanced in this paper are not supported by more detailed information on the case reported. No mention is made of blocking or "developing" antibodies, as contrasted with agglutinating antibodies, in the serum of either the mother or child.

L. E. Y.

CHRONIC HAEMOLYTIC POLYCYTHAEMIA. *L. Rau, R. J. Pulvertaft, and J. G. Hamble*. Proc. Roy. Soc. Med. 39: 307-308, 1946.

This report concerns a 37 year old woman with a five year history of fatigue, dyspnea, blue spots on the face and legs, and ulcers of the lower legs. Physical examination revealed cyanosis, heparosplenomegaly, and several small ulcers of the lower third of both legs. The blood counts were as follows: RBC 6.5 M., HGB 140 per cent, WBC 9,200 (P60, L30, M4, E4), platelets 95,000. The serum bilirubin was 1.6 mg. indirect, and the reticulocytes numbered 3 per cent. The blood smear showed spherocytosis, and in hypotonic solutions hemolysis began at 0.55 per cent sodium chloride and was complete at 0.33 per cent. The bone marrow was hypercellular and showed marked normoblastic hyperplasia, with 5.6 per cent of the red cells in mitosis. The ratio of normoblasts to granulocytes was 3:1.

To the authors this case is easily explained. They consider the underlying disease to be a hemolytic process. Most cases of hemolysis are accompanied by anemia, but in this individual, they state, hyper-

compensation by the bone marrow resulted in the development of erythrocytosis. Certainly such an event is rare. It is difficult to see why there is not a total bone marrow reaction, for the white and platelet counts are not elevated; but, on the other hand, this is not the typical picture of polycythemia vera with hemolysis, for the same reason (i.e., the reaction is purely erythrocytic). The possibility that this is an example of symptomatic hemolytic anemia in association with polycythemia vera, however, cannot be ruled out, for a certain number of polycythemia cases do not show leukocytosis and thrombocytosis. The occurrence of leg ulcers, seen in other chronic hemolytic processes, is of interest. The case is presented as a curiosity of obscure pathogenesis.

S. E.

DISCUSSION ON THE LIFE AND DEATH OF THE RED BLOOD CORPUSCLE. *S. T. Callender and J. F. Loutit*. Proc. Roy. Soc. Med. 34: 755-760, 1946.

Callender summarizes her observations on the length of survival of transfused erythrocytes in the circulation of normal male and female subjects as determined with the Ashby technic. In male subjects the average life of the transfused red cells was 60 days and the rate of destruction was 0.83 per cent of the initial amount per day. This indicated that the red blood cells live nearly a constant time—120 days from their birth. The average life after transfusion is half this—60 days, since the transfused cells are of all ages and have already lived 60 days on the average. In females menstrual loss produced different types of survival curves, although the life of cells was 120 days, just as in males.

Loutit discusses the survival of erythrocytes transfused into patients with various hematologic abnormalities, survival being determined by the Ashby technics. Normal cells transfused into patients with hypochromic anemia had a life span of 100-105 days.

Normal erythrocytes transfused into patients with pernicious anemia showed an average survival of 40-5 days (in contrast to the average survival of 60 days in normal recipients), a low value which probably is attributable to the fact that the transfused blood had been stored for 5-14 days. Transfusion of blood from pernicious anemia patients into normal subjects was followed by rapid disappearance of the transfused cells from the recipient's circulation. Fifty per cent survival occurred at the 10th and 12th days. These observations suggest that the cells of patients with pernicious anemia are destroyed a great deal more rapidly than normal.

Transfusion of normal cells into patients with familial hemolytic anemia was followed by a normal survival curve (maximum 120 days). In marked contrast when normal cells were transfused into cases of acquired hemolytic jaundice, they were destroyed very rapidly, the mean 50 per cent survival being 5 days (in contrast to 34 days in familial hemolytic anemia).

When cells from patients with familial hemolytic anemia were transfused into normal subjects they were rapidly destroyed, 50 per cent survival being noted between the 4th and 15th days. Cells of acquired hemolytic anemia transfused into normal recipients survived normally (50 per cent survival in excess of 50 days).

These observations are interpreted as indicating that the cells of familial hemolytic anemia are abnormal due to an inborn defect of the cell, while in acquired hemolytic anemia the cells are "sensitized" by some circulating hemolysin.

J. F. R.

THE DISAPPEARANCE OF SULPHAEMOGLOBIN FROM CIRCULATING BLOOD IN RELATION TO RED CELL DESTRUCTION. *E. M. Jope*. Proc. Roy. Soc. Med. 39: 760-762, 1946.

Jope studied spectrophotometrically the disappearance of methemoglobin (MHB) and sulfhemoglobin (SHb) from the circulating blood of 7 TNT workers after their withdrawal from contact with TNT. The plots of SHb levels against time after removal from contact with TNT were well fitted by a straight line indicating complete disappearance of SHb at  $116 (\pm 5)$  days. This was considered a valid estimate of the life span of red cells containing SHb, upon the assumptions that (1) the intact red cell has no means of transforming SHb and the body no means of removing it other than by removal of the red cells which contain it, (2) no significant SHb formation continues after removal from TNT, and (3) neither SHb nor the heme portion of its molecule is incorporated into new cells. Jope points out that in relating the destruction of red cells containing SHb to that of normal cells it must also be assumed that (4) SHb is formed at random in cells of all ages, and (5) formation of SHb within the cell neither

prolongs nor shortens its life. All of these assumptions seem reasonable to the author except that some formation of SHb may continue for a few days after removal of the toxic agent.

Conclusions as to average life span of erythrocytes containing SHb and the linear decay curve agree well with the results obtained by differential agglutination (Ashby) studies and  $N^{15}$  isotope methods. SHb and  $N^{15}$  isotope methods have the advantage of permitting studies on the longevity of the subject's own cells.

In contrast to SHb, MHB disappeared from circulating blood within 2 to 5 days after removal of the causative agent.

Other aspects of Jope's work have been published in the *Brit. J. Industr. Med.* 3: 136, 1946 (see following abstract).

L. E. Y.

DISAPPEARANCE OF SULFHEMOGLOBIN FROM THE BLOOD OF T. N. T. WORKERS IN RELATION TO THE DYNAMICS OF RED CELL DESTRUCTION. E. M. Jope, *Brit. J. Industr. Med.* 3: 136-142, 1946.

Methemoglobin and sulfhemoglobin were found in the blood of TNT workers. After removing the patients from all further occupational exposure, the disappearance rate of these two pigments from the circulating blood was determined spectrophotometrically. Methemoglobin was gone in less than 2 weeks. However, in seven workers with an original amount of over 4 per cent sulfhemoglobin, there was a straight line disappearance curve of the latter pigment, extending over 110 to 120 days.

This disappearance curve corresponds to the survival of a mixed age population of erythrocytes studied by the agglutination technics. It was concluded that once formed in the cell, sulfhemoglobin remained there until the cell was destroyed. The abnormal pigment formation did not affect the viability of the erythrocytes.

C. A. F.

STUDIES OF THE FORMATION OF HEME AND ON THE AVERAGE LIFE TIME OF THE HUMAN RED BLOOD CELL. D. Shinn and D. Rittenberg, *Fed. Proc.* 5, 153, 1946.

Investigations of the life span of the red cells have come into prominence in recent years. The usual methods of study have been based upon the study of transfused red cells by the Ashby technic. In the present note, the authors have approached the problem from a different point of view. They have found that feeding glycine labeled with an isotope of nitrogen,  $N^{15}$ , results in the production of a heme most of whose nitrogen is of this isotopic form. Quantitative studies led to the conclusion that glycine is the nitrogen precursor of the protoporphyrin of hemoglobin; various other materials (proline, leucine, glutamic acid, ammonium citrate) did not substitute for glycine in heme formation. These workers, accordingly, could follow the concentration of the isotope in the red cells for months after labeling with  $N^{15}$ ; and they found that the average life of the red cell, by this technic, was about 125 days. This result correlates well with those obtained by the transfusion-Ashby technic.

S. E.

## IRON AND BLOOD PIGMENT METABOLISM

STUDIES IN IRON TRANSPORTATION AND METABOLISM. V. UTILIZATION OF INTRAVENOUSLY INJECTED RADIOACTIVE IRON FOR HEMOGLOBIN SYNTHESIS, AND AN EVALUATION OF THE RADIOACTIVE IRON METHOD FOR STUDYING IRON ABSORPTION. R. Dubach, C. V. Moore, and V. Minnich, *J. Lab. & Clin. Med.* 31: 1201-1222, 1946.

The appearance of injected radioactive iron in circulating hemoglobin, in the excreta, and in various tissues was studied in normal human subjects, patients with various types of anemia, and in dogs rendered anemic by hemorrhage and by the administration of phenylhydrazine.

In normal human subjects the injected iron appeared in the circulating hemoglobin with great rapidity, more than 60 per cent being present in the circulating red cells seven days after the injection. Eventually 80 to 110 per cent of the injected dose was reported as appearing in the circulating red blood cells. The authors' values for the percentage utilization of the iron may be criticized since these values are derived from an assumed blood and cell volume. The blood volume was arbitrarily assumed to be 80 cc. per kilogram body weight, and the red blood cell mass presumably was calculated from this value and the venous hematocrit without further correction.

It is rather generally recognized that blood volume may vary widely from these arbitrary figures from

one normal individual to another, and that in anemic states marked variations occur. This criticism is recognized by the authors, but its importance is deprecated. Furthermore, it is also probable that the actual circulating erythrocyte mass is considerably less than the values calculated from the assumed blood volume and the venous hematocrit. These considerations would suggest that the amount of iron actually appearing in the circulating cell mass might be considerably less than the values given by the authors.

Normal dogs utilized injected iron less rapidly and less completely than the normal human subjects. The difference in total utilization conceivably might be related to errors introduced by assuming a constant 80 cc. blood volume for both human and animal subjects.

Tissue analyses were made to determine the distribution of radioactive iron in 2 dogs. Seventy-two and 78 per cent of the injected material was accounted for, most of it being present in circulating hemoglobin, bone marrow, and liver.

Utilization of iron was more rapid and more complete in iron-deficient human patients and dogs. Utilization was very slight in patients with hypoplastic anemia. In pernicious anemia utilization was slight during relapse, but was increased following injection of liver extract, indicating that some of the iron had temporarily been deposited in storage depots.

In hemolytic anemias utilization was less than normal and there was apparent rapid fluctuation in radio iron concentration in the circulating cell mass, fluctuations attributed by the authors to rapid hemolysis of the subjects' erythrocytes. Part of the hemoglobin iron released from hemolyzed erythrocytes was rapidly reutilized for formation of new hemoglobin.

The authors present a justified criticism of the use of the appearance of orally administered radioactive iron in circulating erythrocyte hemoglobin as an indication of iron absorption. They point out that appearance of iron in the circulating erythrocytes is determined by rate of erythrocyte formation as well as by the amount of iron absorbed from the bowel.

J. F. R.

DETERMINATION OF CARBON MONOXIDE IN BLOOD AND OF TOTAL AND ACTIVE HEMOGLOBIN BY CARBON MONOXIDE CAPACITY. INACTIVE HEMOGLOBIN AND METHHEMOGLOBIN CONTENTS OF NORMAL HUMAN BLOOD. D. D. Van Slyke, A. Miller, J. R. Weissger, and W. O. Cruz. J. Biol. Chem. 166: 121-148, 1946.

The authors describe improved technics for determination of total hemoglobin, active hemoglobin, and inactive hemoglobin by CO capacity procedures. The accuracy of the methods in their hands was considered to be for total hemoglobin  $\pm 0.26$  per cent, active hemoglobin  $\pm 0.38$ , and inactive hemoglobin  $\pm 0.42$  (probable error calculated as  $0.674 \times$  standard deviation).

In human blood the mean inactive hemoglobin by the CO method was 1.3 per cent  $\pm 0.35$  per cent of total hemoglobin when the analysis was performed immediately after drawing blood. This fell to 0.56 per cent  $\pm 0.23$  per cent in 2-4 hours. Mean methemoglobin by the spectrophotometric method of Horecker and Brackett was only 0.4 per cent of total hemoglobin and there was no change over 2-4 hours in this value.

The results presented a problem. It appeared that 1 per cent of total hemoglobin (as measured by the  $\text{Na}_2\text{S}_2\text{O}_4$ -CO method) existed in an inactive form, resembling methemoglobin in its ability to bind CO only after reduction of  $\text{Fe}^{+++}$  to  $\text{Fe}^{++}$ , but differing from methemoglobin in not showing the methemoglobin color reaction with HCN, and in rapidly acquiring ability to combine with CO as blood stood *in vitro*.

C. A. F.

## LEUKEMIA AND LYMPHOMA

STUDIES ON THE LEUKOCYTIC PICTURE IN BRUCELLOSIS. M. R. Castaneda and G. Guerrero I. J. Inf. Dis. 78: 42-48, 1946.

The authors report an analysis of the white blood count in a series of 888 cases of brucellosis seen in Mexico. In most of the cases the causative organism was *Brucella melitensis*. The infection was acute in half the cases (present for less than three months), and chronic in the other half (present for three to twelve or more months). A single white and differential count taken from each patient was the basis for the study.

In 30 per cent of the cases leukopenia was present (2,500 to 5,500 white blood cells per cu. mm.). In 52 per cent of the cases the white count ranged from 6,500 to 9,500; and in the remaining 18 per cent the white count was between 10,500 and 20,000. In the cases with leukopenia there was relative lymphocyto-

sis and therefore neutropenia. In the cases with normal or high white counts, the percentage of lymphocytes was correspondingly high, so that absolute lymphocytosis was present in most of these cases. Neutropenia occurred in the cases with low and normal white counts, but when the counts became high, absolute polymorphonuclear leukocytosis was also present.

As a result of their data (many of which do not appear in the published report), the authors conclude that the usual concept that brucellosis is commonly associated with leukopenia is at fault. Brucellosis, rather, is a lymphocytogenic disorder, and is usually associated with a normal or high white count with absolute lymphocytosis. In the cases in which neutropenia does occur, the authors believe that it is probably the result of inhibition of the bone marrow by some material produced in a damaged spleen. Critical evaluation of these conclusions is not possible from the data presented. The choice of normal values by the authors is somewhat unorthodox: they consider a white count of 5,500 as leukopenia, and 6,500 questionably normal. The normal lymphocyte count is considered to be 2,800, whereas a range of 1,500 to 3,000 is more commonly maintained. What data are given, however, suggest that in fully 20 per cent of their cases there is a definite leukocytosis, a fact at variance with the commonly accepted data S. E.

ELECTROCARDIOGRAPHIC EVIDENCE OF CARDIAC COMPLICATIONS IN INFECTIOUS MONONUCLEOSIS. IV. F. Evans and A. Graybiel. *Am. J. Med. Sc.* 211: 220-226, 1946.

Four cases of infectious mononucleosis are reported in which there was clinical or electrocardiographic evidence of involvement of the heart. The prominent electrocardiographic change was flattening and inversion of the T-waves. In 2 cases a friction rub was audible over the heart. All cases showed return toward normal after subsidence of the infection, although alterations in the T-waves often persisted for a time after the patient was clinically well.

The authors believe that the cardiac changes were indicative of pericardial rather than myocardial involvement. The incidence of these changes in the authors' series was approximately 4 per cent.

S. E.

HEART COMPLICATIONS IN INFECTIOUS MONONUCLEOSIS. F. J. Geraghty. *South. M. J.* 39: 693-696, 1946.

The author reports a 14 year old boy with infectious mononucleosis whose electrocardiogram showed marked changes in the Q- and T-waves during the height of the disease, with a gradual return to normal after subsidence. He gathers six other reports of cardiac involvement in infectious mononucleosis from the literature, including prolongation of the PR-interval, alterations of the T-wave, and occurrence of premature ventricular contractions. In one other case the diagnosis of mitral stenosis was made on clinical grounds, and in yet another a rheumatic valvular lesion was found at autopsy.

Geraghty considers the disorder to be an acute granulomatous process with reticulo-endothelial proliferation, mononuclear infiltration, and necrosis. Involvements of the central nervous system, kidneys, lymph nodes, gastrointestinal system, and skin are well recognized in the disorder. Involvement of the heart may correspondingly be expected to occur, and usually, he believes, consists of myocardial changes which show up on the electrocardiogram as similar to those usually associated with various acute infections.

S. E.

PRIMARY SPLENIC GRANULOCYTOPENIA AND LYMPHOPENIA. H. Levy. *Proc. Roy. Soc. M.* 39: 299-300, 1946.

A case of hepatosplenomegaly and leukopenia is presented. Despite the reduced numbers of granulocytes in the circulating blood, the bone marrow showed active granulocytopoiesis. The fact that the patient showed polyarthritic changes suggestive of rheumatoid arthritis makes it likely that the disorder was an example of Felty's syndrome (rheumatoid arthritis with splenomegaly and leukopenia), rather than truly primary splenic neutropenia.

The author draws attention to the fact that splenomegaly may be associated not only with neutropenia but also with lymphocytopenia. In this instance, of a total of 900 to 1,750 white cells per cu. mm., only 80 were granulocytes (normal over 3,000) and 500 to 1000 were lymphocytes (normal over 1,500). The occurrence of lymphocytopenia in such instances suggests a relationship between the enlarged spleen and the lymphocytes or their sites of production in the body, similar to the postulated relationship between the spleen and granulocytes or bone marrow.

No splenectomy was done in this case. Pyridoxine had no effect on the blood counts.

S. E.

THE BONE CHANGES OF LEUKEMIA IN CHILDREN. B. S. Kalayjian, P. A. Herfert, and L. A. Erf. *Radiol.* 47: 223-233, 1946.

The authors describe 2 cases of lymphoblastic leukemia in children in which the presenting complaints were bone or joint pains. In the first case migratory polyossalgia was associated with fever, night sweats, increased sedimentation rate, and a leukocytosis but with a normal differential count. In the second case polyarthralgia and actual objective joint changes were present, and the blood count showed absolute lymphocytosis. In both instances the bone marrow was infiltrated with blast forms, and the diagnosis of lymphoblastic leukemia was confirmed at autopsy.

X-ray examination of the bones in both patients showed changes which were considered diagnostic of leukemia. According to the authors, these changes are as follows: (1) patchy destruction of bone; (2) expansion of the marrow cavity, with resultant atrophy of the cortex; (3) elevation of the periosteum by leukemic infiltration, with formation of new bone in lamellae parallel to the shaft of the bone; and (4) demineralization. Pathologically, these changes are found to be due largely to infiltration of bone marrow, cortex, and periosteum with leukemic tissue.

These x-ray changes are not specific for leukemia, and in most cases the diagnosis is made independently of x-ray examinations. The authors suggest, however, that the persistence of bone or joint pain in a child, which does not respond to the usual treatment with salicylates, sulfonamides, and penicillin, should call for x-ray examination of all the bones (especially the long bones), and consideration of the possibility of leukemia.

S. E.

BASOPHILIC LEUKEMIA. A. E. Carty, T. E. Nettles, and E. H. Hidden. *South. M. J.* 39: 325-332, 1946.

This paper reports a patient with chronic leukemia in whom the outstanding circulating white blood cell was the basophil. The patient's initial complaint was enlargement of the abdomen, which was found to be the result of massive splenomegaly. Treatment with x-irradiation resulted in moderate improvement and some reduction of the white blood count. Four months before death the white count was 50,400, and the blood smear showed adult neutrophils, basophils, and eosinophils, as well as neutrophilic, eosinophilic, and basophilic myelocytes. Granulocytes totaled 92 per cent of the circulating white cells, of which only 8 per cent were basophilic. A few days before death the white count was 16,600; there were 86 per cent granulocytes of all types; and there were 6 per cent basophilic myelocytes and 66 per cent adult basophils in the circulating blood. Postmortem examination showed leukemic infiltration, mostly with myeloblasts, of spleen, liver, pancreas, bone marrow, lungs, heart, kidneys, and stomach.

The authors find nine reports in the literature of true leukemia confirmed by autopsy, in which a basophilia of over 25 per cent was noted. In most of these cases the data were obtained only terminally. The authors consider that basophilic leukemia is not a disease entity unto itself; rather, that the basic disorder is myelogenous leukemia, and that the basophilia is merely an unexplained terminal event in certain rare instances of chronic myelogenous leukemia.

S. E.

RADIATION TREATMENT OF LOCALIZED MALIGNANT LYMPHOMA. G. V. Holmes and M. D. Schulz. *New England J. Med.* 235: 789-792, 1946.

Records of 500 patients with malignant lymphoma were reviewed. Only those patients were considered whose lesion was localized and whose treatment was limited to x-ray. Fifteen cases were found whose diagnosis was established by biopsy who were alive and apparently free of the disease five years later. The cases included, according to the classifications of Gall and Mallory (*Am. J. Pathol.* 18: 381-429, 1942), two stem-cell, one clasmatoxenic, three lymphocytic and one Hodgkin's lymphoma, one Hodgkin's sarcoma, and one follicular lymphoma. X-ray dosage was from 1000 to over 2000 r.

According to Gall and Mallory, about 10 per cent of lymphomas are localized at autopsy. Gall (*Ann. Surg.* 116: 1024, 1943) reported a group of about 16 patients, if the same criteria are used, who were alive and free from their disease after surgical excision. Both studies provide valuable data relating to the prognosis of localized lymphoma, with these different forms of treatment.

C. A. F.

# BOOK REVIEWS

*Clinical Hematology.* By MAXWELL M. WINTROBE. Lea & Febiger, Philadelphia, 2nd edition, 1946.

This second edition of Wintrobe's book published four years after the first is distinguished first of all by being so remarkably up to date that much of the material has not hitherto been published. Advances of only a month or so ago are noted and duly charted. There are, for example, complete discussions of folic acid therapy, the Rh factor and its importance in hemolytic disease of the newborn, the use of the nitrogen mustards in Hodgkin's disease and related diseases, the anemia of infection.

Even more than before, the book is the best single text of hematology in the English language. Its only rival, Naegeli's classic in German, is now outdated. Furthermore, it does not have Naegeli's dogmatism. The discussions are complete and well annotated with an unusually thorough bibliography, making the book highly acceptable, not only to the student but to the clinician and investigator. Charts, photomicrographs, and figures have been increased in number and greatly improved in quality over those of the first edition. The sections on sickle cell anemia and Mediterranean anemia ("thalassemia") are unusually good.

One of the few criticisms the reviewer finds with this otherwise remarkable book is in the matter of classification. Since anemia is designated as either macrocytic, normocytic, or microcytic, the hemolytic anemias, for example, are classified under the designation of the "normocytic anemias." As far as possible, it would seem more desirable to group the anemias on an etiologic basis. Thus they could be classified as (1) due to a deficiency of blood-building materials, (2) due to a disturbance in the bone marrow, or (3) due to excessive blood loss, whether by hemorrhage or undue hemolysis. Another possible criticism is in the use of Rhoad's term "refractory anemia" for those cases of anemia usually with leukopenia and thrombocytopenia, which fail to respond to liver extract, iron, and the vitamins. The brilliant results obtained with folic acid in some of these cases, the response to splenectomy in others, would seem to indicate that what was "refractory" yesterday may certainly not be so today. The 860 odd pages are crammed with information which is completely sound, up to the minute, and unusually well presented. The whole makes a volume which should be a *must* in every hospital medical library and on the shelves of the forward-looking physician.

*El Diagnostico por la Punción Esternal.* By JERÓNIMO FORTEZA BOVER, M.D. Ediciones Morata, Madrid, 1946. Pp. 317. 90 Pesetas.

This is an excellent monograph on the sternal puncture, its technic, and the results obtained from study of clinical hematologic material. The book is well written and is illustrated by numerous photomicrographs and colored plates. Of the numerous monographs on this subject, this seems to the reviewer one of the soundest. There is a nice intermingling of straight histology with such practical aspects as differential diagnosis of the various anemias, leukemias, leukopenias, and thrombocytopenias. An example of excellent description and sound judgment is the chapter, "Sternal Puncture in the Diagnosis and Differential Diagnosis of the Hemorrhagic Purpuras." The megakaryocytic changes in idiopathic thrombocytopenic purpura are excellently depicted and in keeping with recent observations (cf. Blood 1: 27, 1946). The differential diagnosis of the splenomegalies with particular reference to the sternal marrow puncture is systematically discussed. There is an excellent bibliography of about 600 complete references. Since the Spanish text is eminently simple the book can be heartily recommended even to English-reading students.

# BLOOD

## *The Journal of Hematology*

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### THE ASSOCIATION OF THE GUILLAIN-BARRÉ SYNDROME WITH INFECTIOUS MONONUCLEOSIS

WITH A REPORT OF TWO FATAL CASES

By WALTER RICKER, LT. COL., M.C., A.U.S., ALFRED BLUMBERG, COL.,  
M.C., A.U.S., CLIFFORD H. PETERS, CAPT., M. C., A.U.S., AND  
ARNOLD WIDERMANN, CAPT., M. C., A.U.S.

INFECTIOUS mononucleosis associated with the Guillain-Barré syndrome has been reported but once.<sup>1,2</sup> In this case the combination of diseases did not prove fatal. The Army Institute of Pathology has in its files the records of 36 fatal cases of Guillain-Barré syndrome, the data from 30 of which are being reported by Kernohan and Haymaker,<sup>3</sup> and of 9 fatal cases of infectious mononucleosis which form the basis of a study by Custer and Smith.<sup>4</sup> There were 2 instances in which both Guillain-Barré syndrome and infectious mononucleosis were encountered. These 2 cases, the first of their kind to terminate fatally, to judge from published reports, form the basis of the present paper.

#### REPORT OF CASES

The clinical and pathologic observations on these 2 fatal cases will be detailed briefly to show that they exhibited characteristic manifestations of both Guillain-Barré syndrome and infectious mononucleosis.

#### Case 1 (A.I.P. Acc. 151166)

*Clinical Data.* A 22 year old, white male was admitted to the hospital on September 19, 1945, complaining chiefly of headache and fever. A moderately severe headache had begun 11 days before admission, but the patient had remained on duty. Several times during this period he had had chilly sensations, aches in his elbow and knee joints, and fever. His past history and family history were noncontributory.

On physical examination the patient appeared moderately ill but in no great distress. Temperature was 102° F., pulse 86, and respirations 20. The only other positive finding was a slight enlargement of the lymph nodes in the anterior cervical chain. Examination of the blood showed the following: hemoglobin 15.5 Gm. per cent, erythrocytes 4.4 million, leukocytes 10,200, with lymphocytes 82 per cent, neutrophils 15 per cent, eosinophils 1 per cent, and monocytes 2 per cent. Many of the lymphocytes were reported as atypical and their structural characteristics were suggestive of infectious mononucleosis; in view of these findings a tentative diagnosis of infectious mononucleosis was made.

Thirty-six hours after the patient's admission, blood smears and a blood specimen for the determination of heterophile antibody reaction were submitted to the command laboratory. The report received after the death of the patient revealed that the blood smears were "so characteristic as to be diagnostic of infectious mononucleosis", the heterophile antibody reaction was positive in a dilution of 1:125.

From the Army Institute of Pathology, Washington 25, D. C.

On the second day the patient complained of increasingly severe headache, and moderate nuchal rigidity became apparent. Spinal fluid was obtained under normal pressure; it was clear and contained 9 cells per cu. mm., of which 94 per cent were lymphocytes and 6 per cent polymorphonuclear leukocytes. Sugar was present in normal amount. The protein content was increased. No micro-organisms were found in smears, and cultures remained sterile.

On the third day the patient complained of tingling sensations and numbness in his hands and feet. Neurologic examination showed a complete right facial paralysis of peripheral type, moderate depression of the gag reflex with some difficulty in swallowing, hypesthesia in both hands and feet, and flaccid paralysis of muscle groups of all four extremities with absence of triceps, biceps, patellar, and ankle reflexes. Respiratory movements were accomplished with difficulty. There was no involvement of the abdominal musculature or of the rectal or urinary sphincters. His sensorium remained clear, although he became somewhat apprehensive. The picture was essentially one of an extensive, rapidly progressing, peripheral neuropathy, associated with lymphocytic meningitis.

By the fifth day after admission dysphagia and respiratory paralysis had developed. The patient was placed in a respirator. Spinal fluid taken at this time was clear and contained 36 cells, of which 98 per cent were lymphocytes and 2 per cent neutrophils; total protein was 81 mg. per cent.

Respiratory paralysis became more marked, and following a sudden clonic convulsion the patient died on September 26, seven days after admission to the hospital.

*Postmortem Observations.* The pertinent pathologic changes were limited to the nervous system, spleen, and lymph nodes.

*Nervous System.* The brain weighed 1520 Gm. The vessels in the leptomeninges, choroid plexus, cerebellum, pons, medulla oblongata, and spinal cord were engorged, and there were diffuse petechial hemorrhages. On cross section of the cord the left anterior horn of the dorsal region appeared pale. The central nervous system was otherwise grossly normal. Peripheral nerves beyond the confines of the spinal canal were not examined.

Microscopically, the leptomeninges were congested, showed occasional minute hemorrhages, and in the arachnoid there were moderate numbers of mononuclear cells. Occasional small perivascular hemorrhages were encountered in the cerebrum, most prominently in the hippocampal region. In the cerebellum the Purkinje cells were considerably disorted, many nuclei being either indistinct or indiscernible. In the left anterior horn of the dorsal cord the motor cells showed peripheral condensation of the Nissl substance and nuclear chromatin, and vacuolization of the cytoplasm. Otherwise there were no changes in the spinal cord. In the interfascicular perineurium, particularly related to blood vessels of an anterior nerve root, there were accumulations of small round cells, scattered larger mononuclear cells, and a few eosinophils (fig. 1).

*Spleen.* The spleen weighed 760 Gm. and presented a tense capsule. The pulp was soft, the central portion being almost diffusent. Microscopically, the capsule was moderately "infiltrated" with large mononuclear cells showing folded, indented, or partially lobulated nuclei with fine chromatin. The cytoplasm was usually basophilic and often appeared finely vacuolated. Similar mononuclear cells were sprinkled through the trabeculae, and large accumulations of them were present in the subendothelial spaces of the trabecular veins and within the adventitial meshes of the trabecular arteries (fig. 5). The splenic follicles were present in the usual numbers and were of normal size or smaller. The red pulp was highly cellular, apparently representing a hyperplastic process of the intrinsic, cellular elements. Lying as free cells throughout the pulp were many of the large, atypical mononuclears.

*Lymph Nodes.* The lymph nodes were shotty, firm, and discrete. All sections showed the same picture: hyperplasia of the extrafollicular parenchyma resulting in blurring of the normal architecture. Numerous large mononuclear cells were encountered among the hyperplastic elements of the medulla. The germinal follicles were small, compressed, and partially obliterated.

The *pericardium* and *heart* were essentially normal except for scattered subepicardial petechiae and slight coronary arteriosclerosis. The cross striations of some fibers were indistinct.

The *lungs* were emphysematous and showed hypostatic congestion. Microscopically, most of the alveoli contained large, pigment-filled phagocytes, lymphocytes, erythrocytes, and lobulated mononuclear cells of the variety observed in the spleen and lymph nodes.

The *liver* weighed 2600 Gm. Microscopically, the portal areas were "infiltrated" with numbers of

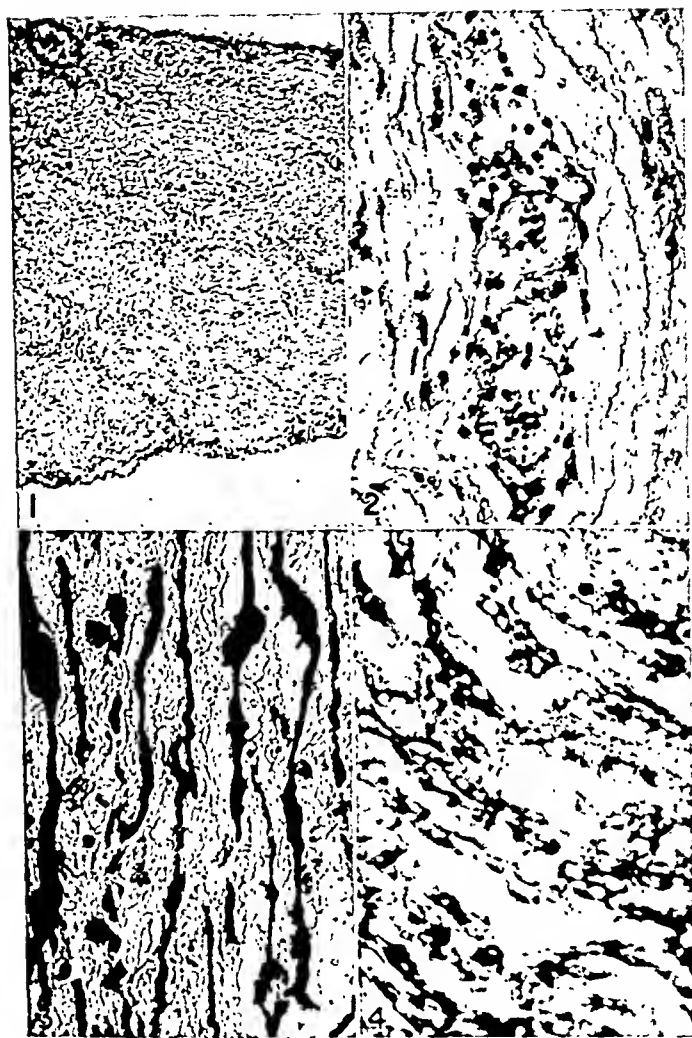


FIG. 1. CASE 1. ANTERIOR ROOT OF SPINAL CORD IN MID-DORSAL REGION

Infiltration of small and large mononuclear cells along periphery of nerve and in the perineurium of the nerve fasciculi, particularly in relation to blood vessels. Hematoxylin and eosin stain.  $\times 100$ . Neg. 93718.

FIG. 2. CASE 2. POSTERIOR NERVE ROOT OF LOWER DORSAL REGION

Small round cells and atypical large mononuclear cells in perineurium related to blood vessels. Distortion of nerve fibers is apparent. Hematoxylin and eosin stain.  $\times 500$ . Neg. 94604.

FIG. 3. CASE 2. CAUDA EQUINA

Irregular swelling, vacuolization, and fragmentation of axis cylinders. Bodian stain.  $\times 650$ . Neg. 94608.

FIG. 4. CASE 2. CAUDA EQUINA

Degeneration of myelin, with irregular droplet formation. Mahon myelin sheath stain.  $\times 1000$ . Neg. 94610.

mononuclear cells of large and small varieties. Immediately beneath the endothelium of the veins of the portal radicles mononuclear cells of the atypical variety were seen, cells showing folding and partial

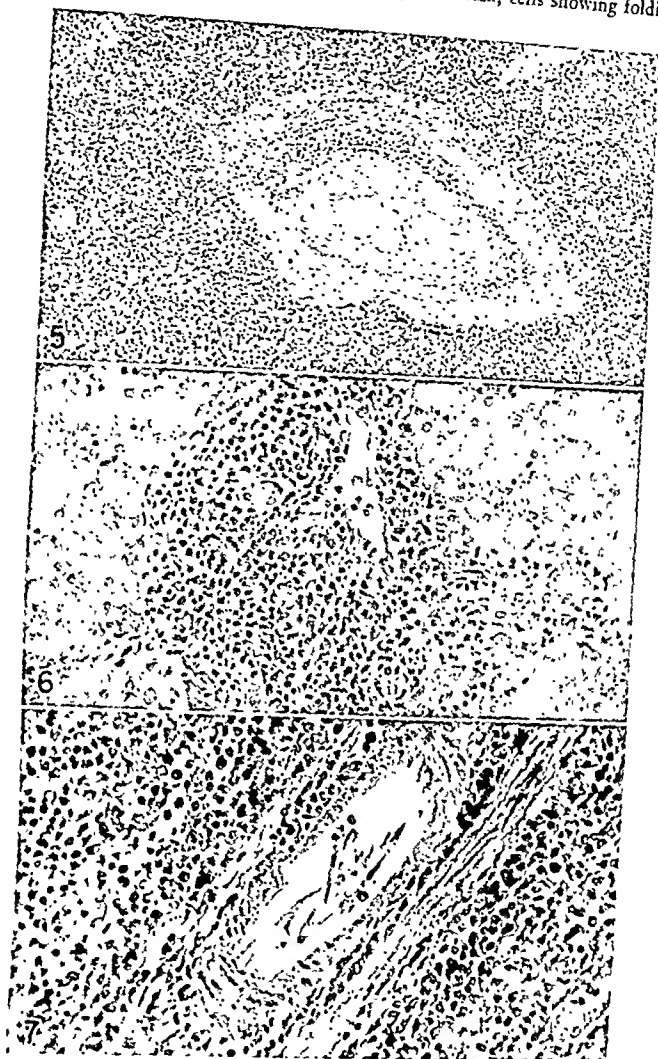


FIG. 5. CASE 1. SPLEEN

"Infiltration" of the adventitia of a trabecular artery. Increased cellularity of the pulp. Hematoxylin and eosin stain.  $\times 100$ . Neg. 93724.

FIG. 6. CASE 1. LIVER

"Infiltration" of the portal zone with mononuclear cells, many of the pale-staining, partially lobulated variety. These cells are present also in the subendothelial space of the portal vein. Hematoxylin and eosin stain.  $\times 400$ . Neg. 94620.

FIG. 7. CASE 2. SPLEEN

"Infiltration" of the adventitia of a small trabecular artery, showing the preponderance of pale-staining, atypical mononuclear cells. Similar cells may be seen in the pulp sinusoids, especially to the right of the trabecula. Hematoxylin and eosin stain.  $\times 355$ . Neg. 94616.

lobation of their nuclei (fig. 6). Occasional cells of the same kind were lying free within the sinusoids of the lobules.

The *kidneys*, microscopically, showed a few of the atypical mononuclears within the stroma.

The *bone marrow* was more cellular than usual, but offered no unusual elements.

The autopsy confirmed the clinical diagnosis of infectious mononucleosis.

#### Case 2 (A.I.P. Acc. 163849)

*Clinical Data.* On November 28, 1945, a 21 year old white male entered the hospital complaining of chilly sensations, headache, weakness, and sore throat. The physical examination was negative except for a temperature of 101° F. The patient was treated symptomatically and returned to duty on December 1. On December 16, 1945, he was readmitted to the hospital because soreness of the throat had not subsided, and he also complained of soreness and weakness in the muscles of the lower extremities. Physical examination showed the pharynx to be reddened, and the cervical lymph nodes were palpable. Examination of the chest and heart was negative except for a loud, blowing systolic murmur at the apex.

Neurologic examination revealed practically no intercostal activity in the upper two-thirds of the chest and only weak intercostal action in the lower third. The diaphragm appeared to be functioning adequately. The patient was able to turn his head from side to side and lift it 2 or 3 inches from the pillow. He was able to move his upper extremities freely, but there was definite weakness. The muscles of the trunk and lower extremities were paralyzed. All deep reflexes, as well as the abdominal and cremasteric, were absent. There were transient paresthesias of the feet, lower legs, and hands, but no objective sensory loss to touch, pinprick, or vibration. He was able to swallow and had no visual disturbances.

Examination of the blood showed erythrocytes 4.7 million, hemoglobin 90 per cent, leukocytes 9,900, of which 39 per cent were lymphocytes, 56 per cent polymorphonuclear leukocytes, and 5 per cent monocytes. The urine was normal. A spinal fluid examination on December 16, 1945, revealed the total protein to be 74 mg. per cent, sugar 50 mg. per cent, chlorides 627 mg. per cent, and there were 8 cells per cu. mm. A throat culture was positive for hemolytic streptococcus. No examination for heterophile antibodies was done.

The patient gradually became dyspneic, and on December 19, 1945, he was placed in a Drinker respirator. Penicillin therapy of 20,000 units every three hours was instituted. Death occurred on December 20, 1945, four days after admission to the hospital.

*Postmortem Observations.* The pertinent pathologic changes were limited to the nervous system, the spleen, liver, and lymph nodes.

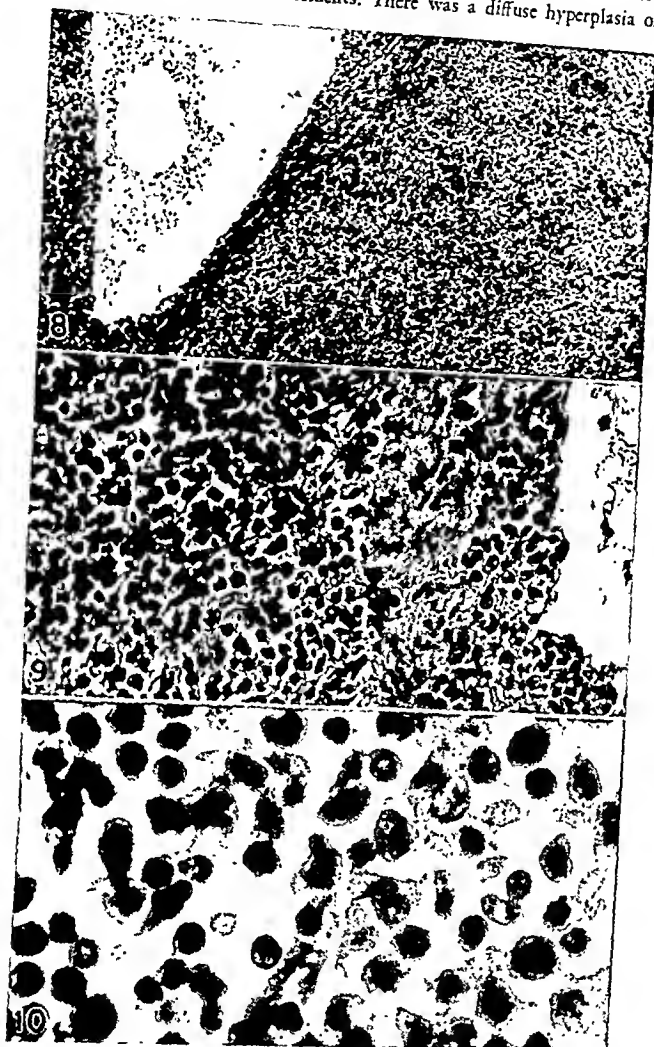
*Nervous System.* The brain weighed 1500 Gm. There was congestion of the capillaries of the leptomeninges, brain, and upper spinal cord, particularly in the gray matter of the upper dorsal segments. The remainder of the cord was grossly normal.

Microscopically, the leptomeninges were edematous with a few small accumulations of lymphocytes, large mononuclears, and occasional neutrophilic leukocytes. The capillaries and small veins in all sections from the central nervous system were engorged. In addition, several small, perivascular hemorrhages were noted in the gray matter of the lower cervical cord. The ganglion cells at all levels of the cord as well as in the cerebral cortex showed mild changes, such as vacuolization, early chromatolysis, decreased affinity of the cytoplasm for eosin, and dispersal of nuclear chromatin. Occasional shrunken ganglion cells, surrounded by an increased number of oligodendroglial satellites, were noted in the cortex of the parietal lobe. Sections from the spinal nerve roots showed marked congestion and edema of nerve fasciculi (fig. 2). Cellular infiltration of the anterior nerve roots was noted at all levels, but was most intense in the cauda equina. Bodian stains revealed marked distortion of the axis cylinders (fig. 3). The myelin sheaths were swollen and disrupted (fig. 4).

Sections of the femoral nerve revealed an infiltrate consisting predominantly of lymphocytes although numerous larger mononuclear elements with distorted nuclei were present. These inflammatory cells were noted particularly about smaller blood vessels. Sections from the facial, acoustic, and phrenic nerves showed slight infiltration. The trigeminal nerve appeared relatively normal.

*Spleen.* The spleen weighed 675 Gm. The capsule was smooth and glistening. On section the pulp was soft, dark red, hyperemic, and bulged over the capsule. The follicles were distinct, the trabecular

markings obscured. Microscopically, the capsule and trabeculae were of normal thickness and moderately "infiltrated" with atypical mononuclear elements. There was a diffuse hyperplasia of the intrinsic,



FIGS. 8 AND 9. CASE 2. SPLEEN

"Infiltration" of the subendothelium of a trabecular vein by large numbers of mononuclear cells, the large, pale-staining variety predominating. Marked cellularity of the surrounding pulp may be seen with similar large cells lying free in the sinusoids, as is particularly evident in figure 9. Hematoxylin and eosin stain. Fig. 8,  $\times 100$ , Neg. 94618. Fig. 9,  $\times 500$ , Neg. 94615.

FIG. 10. CASE 2. SPLENIC PULP

Within the meshes of the increased reticulum elements and in the sinusoids are the large, pale mononuclear cells exhibiting indented, folded, and partially lobulated nuclei. A few, relatively normal small lymphocytes are present. Hematoxylin and eosin stain.  $\times 1360$ . Neg. 94622.

cellular elements of the red pulp. Within the splenic sinusoids large numbers of atypical mononuclear elements, like those described in the preceding case, were encountered (fig. 10). There was moderate venous congestion. The splenic corpuscles were essentially normal. The veins and arteries of the tra-

beculae showed marked, subintimal "infiltrations" with large numbers of the mononuclear cells (figs. 8 and 9), as did also the adventitia of the trabecular arteries (fig. 7).

*Liver.* The organ weighed 2100 Gm. and was grossly normal. Microscopically, the hepatic architecture was adequately preserved, although mild degenerative changes were observed in a few of the parenchymal cells. The predominant change was noted in the portal areas, where a cellular "infiltrate" of lymphocytic and mononuclear type was encountered. Several of the larger veins of the portal spaces showed subendothelial alterations similar to those seen in the trabecular veins of the spleen.

*Lymph Nodes.* Lymph nodes throughout the body were considerably enlarged. They were soft, moist, pale, and homogeneous. Microscopically, the capsules showed slight to moderate mononuclear "infiltration" similar to that in the spleen. The normal architecture was blurred, apparently due to a hyperplasia of the extrafollicular parenchyma. The peripheral sinusoids were unaltered. Scattered throughout the medulla were numerous, atypical mononuclear elements. The germinal follicles were largely obliterated, although a few, small, compressed centers remained (figs. 11 and 12).

The *lungs* were heavy and congested, weighing 1350 Gm. Sections from the lower lobes showed alveoli filled with a homogeneous eosinophilic precipitate containing a few mononuclear and polymorphonuclear leukocytes. The lower trachea and main bronchi were congested and their mucosa and submucosa densely infiltrated with lymphocytes, plasma cells, large mononuclear cells, and neutrophils.

The *heart* weighed 275 Gm. and was grossly normal. Microscopically, an occasional small, perivascular accumulation of lymphocytes was seen in the epicardium of the left ventricle.

The *kidneys* weighed 325 Gm. and presented moist, dark red, hyperemic parenchyma. Microscopically a few small collections of mononuclear cells were noted in the interstitial connective tissue.

The *gastrointestinal tract* revealed no gross abnormality. On microscopic examination the mucosa and submucosa of the pharynx, esophagus, and small intestine were infiltrated focally and diffusely with small and large mononuclear elements. The lymphatic follicles of the ileum as well as other parts of the gastrointestinal tract showed changes similar to those described for the lymph nodes.

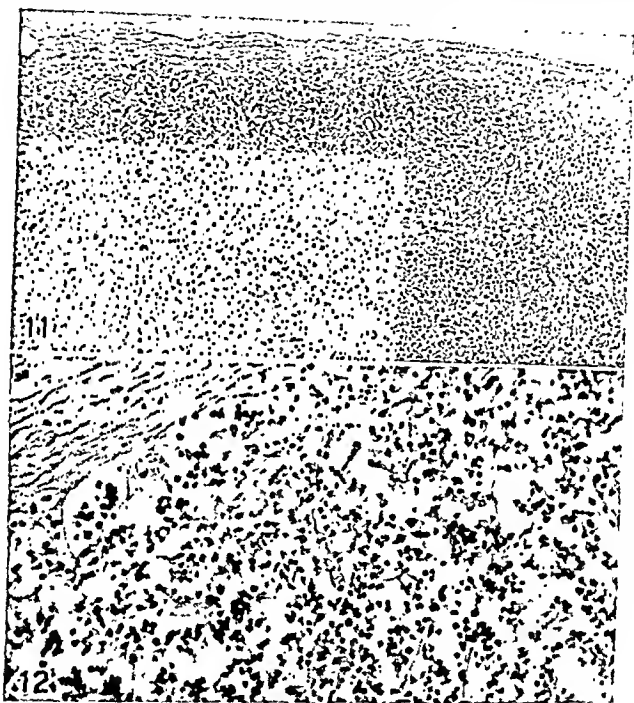
Postmortem blood culture produced no growth. Brain and medulla oblongata were inoculated into mice and guinea pigs but no virus was isolated.

Although serologic confirmation of the diagnosis was never accomplished in this case, the histologic findings were regarded as diagnostic of infectious mononucleosis.

#### DISCUSSION

Involvement of the nervous system, frequently reported in infectious mononucleosis, is usually manifested as lymphocytic meningitis,<sup>6-12</sup> encephalomyelitis,<sup>13</sup> or peripheral neuropathy.<sup>1</sup> However, no previous fatal cases of Guillain-Barré syndrome associated with this disease have been reported. The 1 case reported by Hiller and Fox<sup>1</sup> with the same combination of diseases and subsequent recovery concerned an individual who had enlarged cervical, axillary, and inguinal lymph nodes with enlarged spleen, followed by paralysis of an ascending nature finally involving the facial nerve. The heterophile antibody reaction was positive in a dilution of 1:125. The spinal fluid showed greatly elevated protein but a relatively normal cell count. The patient made a progressive and satisfactory recovery. Furthermore, although visceral lesions in fatal cases of the Guillain-Barré syndrome have been reported,<sup>3, 15-18</sup> none of these have been characteristic of infectious mononucleosis. Sabin and Aring<sup>17</sup> reported 3 fatal cases of Guillain-Barré syndrome in which lesions occurring in the adrenals, liver, kidneys, and heart were described as follows: "The lesions in the adrenals consisted in the main of focal degeneration and infiltration with mononuclear cells; those in the liver of focal cellular infiltration in the capsule and portal spaces, focal necrosis of cells with cellular infiltration, and of focal fatty degeneration; those in the kidneys of focal intertubular infiltration with mononuclear cells, and those in the heart of interstitial infil-

tration with mononuclear and polymorphonuclear cells and in one case of necrosis of isolated muscle fibers and focal phlebitis." In contradistinction to these observations of Sabin and Aring, the visceral lesions in our cases were characteristic of infectious mononucleosis.<sup>4, 19, 20</sup> The splenic changes may be described as follows: (1) moderate cellular "infiltration" of the capsule and trabeculae, (2) hyperplasia of the intrinsic cellular elements of the red pulp with large mononuclear cells in the pulp sinusoids (fig. 10), and (3) certain vascular changes. These vascular changes are first an "infiltration" of the subintima of the trabecular arteries and veins with



FIGS. 11 AND 12. CASE 2. LYMPH NODE

Obliteration of the follicular pattern with increased cellularity of the extrafollicular parenchyma. Moderate infiltration of the capsule may be seen. Preservation of sinusoidal pattern is apparent in figure 12. Hematoxylin and eosin stain. Fig. 11,  $\times 62$ , Neg. 94614. Fig. 12,  $\times 355$ , Neg. 94612.

large atypical mononuclear cells (figs. 8 and 9), and second an "infiltration" of the adventitia of the trabecular arteries with similar cells (figs. 5 and 7). In the experience of Smith and Custer<sup>19</sup> these changes in the spleen are found invariably in infectious mononucleosis, although they may be simulated by other conditions, notably the leukemias and scarlet fever. The lymph nodes showed the typical histologic pattern<sup>4, 20</sup>: infiltration of the capsule, blurring of the architecture with partial obliteration of the lymphatic follicles, and hyperplasia of the extrafollicular parenchyma with numerous, large mononuclear cells encountered within the well preserved sinusoids (figs. 11 and 12). The hepatic changes were largely con-

fined to the portal areas, where a dense "infiltration" of large and small mononuclear elements was seen. A few of the larger portal veins showed subintimal "infiltration" similar to that in the spleen (fig. 6). Although the three organs were most conspicuously the seat of the pathologic changes, small, focal, cellular "infiltrations" were observed in the adrenals, pharynx, trachea, lungs, and in the walls of the gastrointestinal system. The feature common to all of these lesions, as well as to those of the involved nerve tissue, was the large, atypical mononuclear cell, the "leukocytoid" lymphocyte of infectious mononucleosis.

The laboratory examinations of blood smears and serum were diagnostic of infectious mononucleosis in case 1. The lymphocytes were atypical and of the "leukocytoid" variety characteristic of this disease; the heterophile antibody reaction was positive in dilution of 1:1792. In the second case these examinations were not performed, probably because of the rapidly fatal, neurologic progression. However, the lesions were so characteristic of infectious mononucleosis that there was no hesitation in making the positive diagnosis even though these corroborative data were lacking.

#### SUMMARY

Infectious mononucleosis in which death was due to associated ascending paralysis, indistinguishable from that in Guillain-Barré syndrome, was encountered in 2 cases at the Army Institute of Pathology. Study of the data from these cases strongly suggests that infectious mononucleosis is one of the many diseases or agents which may precipitate or give rise to the Guillain-Barré syndrome.

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## CHRONIC NEUTROPENIA

### REPORT OF A CASE NOT CURED BY SPLENECTOMY

By PAUL G. HATTERSLEY, M.D.

THE syndrome of chronic neutropenia has long been recognized. Roberts and Kracke<sup>1</sup> in 1931 described the symptom complex of fatigability, weakness, nervousness, and a predisposition to intercurrent infections which occurred in a large percentage of their patients who had neutropenia. Doan<sup>2</sup> classified the chronic neutropenias in three groups: (1) those cases with a persistently low leukocyte count, but a normal differential, the ability to respond to intercurrent pyogenic infections with a leukocytosis, and with few symptoms; (2) those cases of persistent leukopenia with a disproportionately low granulocyte count, an inability to respond to infection with neutrophilic leukocytosis, and symptoms of weakness and fatigability; (3) those cases of cyclic or recurrent granulocytopenia in which there is repeated recurrence of extreme neutropenia or agranulocytosis, often with severe prostration and mucosal ulceration, alternating with periods of apparent good health and normal blood picture. During the 1930's numerous cases of severe chronic and acute neutropenia were reported which fell into one or another of these categories, as well as some which could not be classified. In many of these cases there was chronic or recurrent exposure to aminopyrine, although the etiologic importance of this agent was not always clear.

In recent years there has been increasing recognition of the importance of the spleen in the neutropenias. The occurrence of some degree of neutropenia, with or without anemia and thrombocytopenia, has frequently been described in such splenomegalic states as Felty's syndrome, Gaucher's disease, Hodgkin's disease, and the congestive splenomegalies, and splenectomy has frequently been followed by remission of the neutropenia.<sup>3,4</sup> In 1938 Reissmann<sup>5</sup> reported the first case of splenic neutropenia associated with splenomegaly for which there was no obvious cause. The sternal marrow was cellular, with an apparent arrest of maturation of granulocytes at the myelocyte level. The neutropenia was promptly cured by splenectomy and the marrow picture returned to normal. The spleen was normal on section. The author postulated an abnormal splenic influence which arrested granulopoiesis in the marrow. The following year Wiseman and Doan<sup>6</sup> briefly reported three cases of "primary splenic neutropenia," all promptly cured by splenectomy. They described hyperplasia of the splenic clasmatoocytes with excessive phagocytosis of granulocytes, and explained the neutropenia on this basis rather than on the basis of any direct splenic effect on the marrow. In a subsequent more extensive report<sup>7</sup> they pointed out that in some cases there are variable degrees of anemia or thrombocytopenia or both accompanying the neutropenia. This they ascribed to excessive splenic phagocytosis of erythrocytes and platelets as well as

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granulocytes, and they likened the situation to that in the hemolytic anemias and in idiopathic thrombocytopenic purpura in which excessive splenic phagocytosis had likewise been observed.<sup>8</sup>

The validity of this interpretation has been questioned by Dameshek.<sup>9,10</sup> His findings in the bone marrow of patients with essential thrombocytopenic purpura before and after splenectomy<sup>11</sup> were interpreted as evidence of an abnormal splenic influence on the marrow which prevented normal maturation of the megakaryocytes and production of platelets. Evidence of such an influence had previously come from the work of Troland and Lee,<sup>12,13</sup> Hobson and Witts,<sup>14</sup> and Rose and Boyer,<sup>15</sup> who had demonstrated a substance in extracts of the spleens of patients with this disease which depressed the platelet count in rabbits. Dameshek suggested, as had Reissmann, that a similar splenic influence on the marrow might be responsible for the neutropenia in splenomegalic states.

A total of 11 case reports of "primary splenic neutropenia" has reached the literature.<sup>3-7,16-20</sup> Whereas in some cases there was evidence of excessive splenic phagocytosis of granulocytes,<sup>6,7,16,19</sup> in others no such evidence could be found, and the picture appeared more suggestive of an abnormal splenic influence on the marrow.<sup>3,17,18,20</sup> All of the reported cases, however, were characterized by definite splenomegaly, and in all there was a prompt and sustained remission of the neutropenia following splenectomy.

In view of the emphasis in recent reports on cure of neutropenia by splenectomy, it is of interest to report a case of severe neutropenia without splenomegaly which was observed for more than ten years, which failed to respond to the usual types of medical therapy, and which was not cured by splenectomy.

#### CASE HISTORY

Mrs. G. A., a 42 year old bookkeeper, entered Stanford University Hospital on January 18, 1946, complaining of an infected puncture wound on the hand of two days' duration.

There was no family history of blood dyscrasias. She had never been very well since childhood, having had a long series of infections of one sort or another from each of which she had recovered very slowly. She had likewise bruised very easily, and on several occasions had bled excessively following tooth extractions or minor surgical procedures. At 13 she had acute rheumatic fever. At 20 a tooth extraction was followed by a streptococcal infection with several weeks of high fever and severe prostration. In her early 20's she began to complain of weakness, fatigability, and headaches, and to have periods of profound collapse. These attacks appeared to be precipitated by hard work or worry and were accompanied by slowly healing ulcers of the mouth. At 26 an induced abortion was followed by persistent pelvic inflammatory disease, from which she was never free of symptoms until hysterectomy and bilateral salpingectomy ten years later.

In 1935, at the age of 32, her first recorded blood count showed a profound neutropenia (leukocytes 5000 per cu. mm., neutrophils 8 per cent) and this observation was repeatedly verified in the ensuing ten years (table 1 and fig. 2). It was discovered at this time that she had for four years periodically been taking moderate amounts of a proprietary headache remedy which contained aminopyrine, as well as empirin compound and moderate amounts of various barbiturates. The use of aminopyrine was immediately discontinued, and during the ensuing ten years she avoided all medications for extended periods, but without remission of her neutropenia. She was likewise given courses of treatment with pentnucleotide, adenylic acid, crude and refined liver extracts, yellow bone marrow, nonspecific proteins, and finally pyridoxine, all without demonstrable effect on neutropenia, symptoms, or susceptibility to infections.

In 1938 a sternal puncture elsewhere yielded marrow which was considered leukemic, and she was subsequently studied in another hospital. Physical examination at that time was reported to reveal the

TABLE I.—*Blood Studies before and after Splenectomy: Case G. A.*

Date	Hemoglobin Gm. per 100 cc.	Platelets per cu. mm.	Leukocytes per cu. mm.	Neutrophils per cu. mm.
7/35	14.6	—	5000	400
11/35	—	—	4600	1400
4/36	—	—	4600	1500
10/36	11.9	—	4100	1200
3/37	—	—	3000	200
12/37	—	—	3200	120
2/38	13.8	—	3300	1320
10/38	11.6	—	2100	270
5/39	—	—	1800	250
9/39	14.5	—	2500	100
4/40	—	—	3000	200
10/40	—	—	3200	960
4/41	—	—	4100	800
10/41	—	—	3400	500
4/42	16.1	188,000	2700	240
10/42	—	—	3200	800
7/43	—	—	2600	1000
3/44	—	—	2500	620
5/45	—	—	1800	200
10/45	—	—	2100	180
1/18/46	14.1	—	1900	250
1/19/46	—	—	1500	120
1/21/46	14.8	142,000	1600	32
1/23/46	15.8	—	1750	35
1/24/46	—	—	2500	275
1/25/46	17.0	152,000	3500	1050
1/26/46	—	—	—	—
Sple- { Morn	—	—	2000	380
nec- { Noon	—	280,000	4700	1645
tomy { Night	15.5	—	2900	174
1/27/46	12.9	395,000	3100	155
1/28/46	—	526,000	4100	492
1/29/46	12.2	934,000	3700	—
1/30/46	—	935,000	4900	147
1/31/46	13.1	—	4800	—
2/ 1/46	—	1,050,000	6400	64
2/ 2/46	14.1	956,000	6000	—
2/ 3/46	—	—	4600	230
2/ 4/46	14.5	—	5400	—
2/ 5/46	—	650,000	4700	376
2/ 6/46	14.3	—	4300	516
2/ 9/46	—	—	3400	408
2/10/46	—	—	3700	—
2/11/46	15.1	845,000	3000	30
2/12/46	—	—	4300	—
2/13/46	13.8	—	2500	50
2/14/46	—	—	3500	—
2/15/46	—	—	2600	156

TABLE 1.—*Concluded*

Date	Hemoglobin Gm. per 100 cc.	Platelets per cu. mm.	Leukocytes per cu. mm.	Neutrophils per cu. mm.
2/23/46	13.1	591,000		
3/1/46	15.5	340,000	3700	407
3/46		186,000	3300	700
4/46	13.0	244,000	2200	570
5/46	15.4	175,000	3000	720
6/46	13.8	114,000	2800	730
7/46	14.7	—	2500	650
8/46	14.7	110,000	4800	—
9/46	15.0	100,000	4000	800
			3000	550

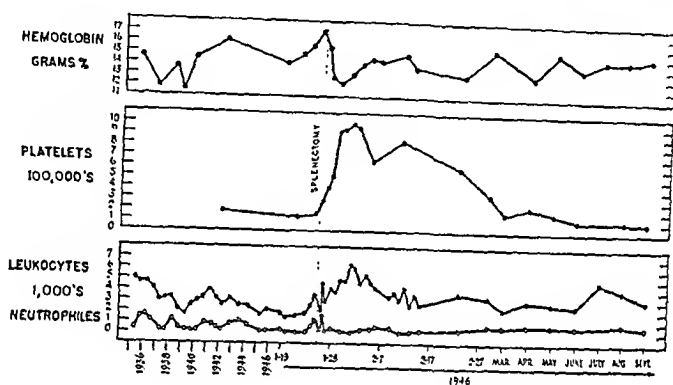


FIG. 1. CASE: G. A. BLOOD STUDIES BEFORE AND AFTER SPLENECTOMY

signs of a mild mitral valvular lesion and a tender mass in her pelvis, apparently inflammatory. Spleen and liver were *not* enlarged, and there was no glandular enlargement. Blood count showed no anemia but a leukocyte count of only 2100 per cu. mm., with 13 per cent neutrophils. Sternal marrow was reported to be cellular with failure of maturation of the neutrophils past the metamyelocyte stage. There was no evidence of leukemia. The diagnosis was "toxic hyperplasia" of the marrow, the impression being that the chronic salpingitis was responsible for the neutropenia.

In the following year a large inflammatory mass was removed from the pelvis, along with the uterus, both tubes and a short segment of bowel. Postoperative convalescence was complicated by protracted wound infection, and except for a transient increase in the leukocyte count immediately following the operation, there was no demonstrable improvement in her blood picture.

During the ensuing six years she had repeated pyogenic infections including furunculosis, paronychia, pyelonephritis, saphenous thrombophlebitis, and repeated severe sore throats, as well as traumatic hematuria following a fall on her left side. Fatigue, depression, bitemporal headaches, and low back ache became progressively worse. She ran a constant low grade fever. At one time she took small doses of sulfadiazine for a urinary tract infection, but this was very soon discontinued. Throughout this period her neutropenia persisted.

In 1942, at the age of 39, she first entered Stanford University Hospital with an upper respiratory infection, bronchitis, and eustachian salpingitis. The only positive physical findings at that time were referable to her current infection. There was still no glandular enlargement or hepatomegaly, and the spleen was not palpable. Pelvic examination revealed no evidence of pelvic abscess. There was no anemia or thrombocytopenia, but the marked neutropenia persisted (leukocytes 2700 per cu. mm., neutrophils

9 per cent). She improved on symptomatic therapy and was discharged without further investigation of her neutropenia.

On January 16, 1946, she broke the skin of her right palm with a rusty nail; before entry into Stanford University Hospital 48 hours later, the site had become red and swollen, and she had developed chills and malaise. Physical examination at that time revealed a well nourished but apprehensive middle-aged woman. Rectal temperature was 38.5° C. There were numerous ecchymoses scattered over the extremities and buttocks, but no petechiae. The eyes appeared normal. The gums were firm, without bleeding, and there were no mucosal ulcers. Ears, nose, and throat were negative. The lungs were clear. The heart appeared slightly enlarged to the left, was regular at a rate of 100 per minute, and a rather soapping first sound was followed by a grade 3 blowing systolic murmur, best heard at the apex. Diastole was clear. The liver and spleen could not be felt and there was no abdominal tenderness. There was a red, swollen puncture wound of the palm of the right hand, with red streaks of lymphangitis on the dorsum of the hand and up the arm, and motion of the fingers was painful. Small tender lymph nodes were palpable in the right epitrochlear and axillary regions, but there was no other adeopathy.

On entry the neutropenia had become more marked, with relative increase of monocytes and eosinophiles, but no primitive leukocytes were found (leukocytes 1500 per cu. mm.; neutrophils 8 per cent, of which 5 per cent were banded forms; eosinophiles 11 per cent; basophiles 1 per cent; lymphocytes 52 per cent; monocytes 28 per cent). There was no anemia, but moderate macrocytosis of the erythrocytes (erythrocytes 3.93 million per cu. mm., hemoglobin 81 per cent Sahli or 13.9 Gm. per 100 cc., hematocrit 42 per cent, M.C.V. 106.9 cu. micra, M.C.H. 32.6 micro-micrograms, M.C.H.C. 33.2 per cent). Red cell fragility was normal. Icterus index was 5, reticulocytes 2.0 per cent. Platelets were somewhat reduced in number (142,000 per cu. mm.) and a few were large atypical forms. Bleeding time was at the upper limit of normal (5 minutes by Duke method), but coagulation time was normal (8 minutes by Lee-White method) and clot retraction was fair. The tourniquet test produced a heavy sprinkling of petechiae. The routine urine and stool examinations were normal, and the Wassermann and Hinton reactions were negative.

Sternal marrow obtained by puncture was extremely cellular, with granulocytes predominating. Myelocytes were considerably increased in number, and there were numerous metamyelocytes and banded neutrophils. No fully mature, segmented neutrophils were found on prolonged search, however, and some of the metamyelocytes and banded neutrophils showed signs of degeneration, with pyknotic nuclei and vacuolated cytoplasm. The megakaryocytes were greatly increased in number, with defective granulation of the cytoplasm and minimal production of platelets at the periphery of the cells. Promegakaryocytes were plentiful and many could be seen to be pinching off bits of their cytoplasm to form atypical, granule-free platelets. There was no infiltration with leukemic or tumor cells and erythroblastic cells appeared normal.

The infection of her hand cleared satisfactorily in a week with penicillin, 30,000 units intramuscularly every 3 hours. Extreme prostration persisted, however, and the neutrophils almost disappeared from the blood (leukocytes 1750 per cu. mm., neutrophils 2 per cent). As the neutropenia had repeatedly failed to respond to medical treatment and constituted a serious menace to her future health, splenectomy was finally decided upon. On January 26, 1946, the spleen and a small piece of liver were removed by Dr. John Menke. The spleen weighed only 150 grams, and no accessory spleens were found. Grossly it appeared normal; microscopically, although there was slight hyperplasia of the endothelial cells lining the sinusoids, no excessive phagocytosis of any cellular elements could be demonstrated in smears or sections. The liver tissue proved to be entirely normal.

Within 1½ hours after operation, the white count had risen appreciably, and within 2½ hours it appeared that a remission of both neutropenia and thrombocytopenia might be in progress (leukocytes 4700 per cu. mm., neutrophils 35 per cent, platelets 280,000 per cu. mm.). A second sternal puncture, just 1½ hours after clamping the splenic pedicle, showed a remarkable change in the megakaryocytes, with increased granularity of their cytoplasm, and evidence that normal platelets were being split off at their periphery. There was no change in the granulocytes, however, mature segmented neutrophils remaining entirely absent in the smears. The marrow picture was subsequently reflected in the peripheral blood, the platelets continuing to climb to a high of over a million on the sixth postoperative day, while the neutropenia reappeared within a few hours, and persisted. A third sternal puncture on the fourth post-

operative day showed further aggregation of platelets around the megakaryocytes, but still no changes in the granulocytes.

The patient's postoperative convalescence was uneventful except for an unexplained bout of gastroenteritis. The surgical wound healed well, and she was discharged on the twentieth postoperative day, feeling weak and tired, but considerably better than on entry. Her neutropenia remained near preoperative levels, however, and has persisted during the eight months of observation since. The platelet count likewise gradually fell to below preoperative levels, and a moderate bruising tendency has returned.

#### DISCUSSION

This case illustrates well the syndrome of severe chronic neutropenia. There was fatigue, listlessness, and weakness which at times amounted to profound prostration. Backaches and headaches were prominent, and there was the emotional instability which has often been described in these cases. There was sustained leukopenia with disproportionate decrease of the neutrophilic cells. There was a marked predisposition to pyogenic infections, and such infections accentuated rather than alleviated the neutropenia. There was a cellular marrow, with an abundance of myelocytes but a paucity of mature neutrophils. In addition there was a defect of platelet formation with asymptomatic thrombocytopenia.

The usual causes of neutropenia appeared to have been eliminated in this case. Repeated negative physical and laboratory examination in the past few years, since surgical treatment of the chronic pelvic inflammatory disease, had failed to indicate the presence of chronic infection. Leukemia was very unlikely in view of the ten year course without anemia, the absence of primitive cells in the peripheral blood, and the lack of bone marrow infiltration. Aplastic anemia similarly was ruled out by the lack of anemia, and the ordinary splenic syndromes by the persistent lack of splenomegaly and of other characteristic signs and symptoms. It is true, there had been exposure to potentially toxic drugs. Aminopyrine was taken for about four years, and it was at the end of this period that neutropenia was discovered. Symptoms apparently had occurred many years before exposure to the drug began, however, and its withdrawal did not effect a cure. Withdrawal of barbiturates and empirin likewise caused no change in the leukocyte level, and the only exposure to sulfonamides was for a brief period, and in very small doses. Since there is no convincing evidence that sensitivity to any of these agents can cause neutropenia which persists for years after they are withdrawn, it seems unlikely that the disease was due to drug therapy.

Despite the persistent lack of splenomegaly, certain features of this case indicated that the neutropenia might be due to some sort of splenic dysfunction. The defect of maturation of granulocytes in the marrow closely resembled the picture described by Reissmann,<sup>5</sup> Nordenson and Roden,<sup>17</sup> and Rogers and Hall<sup>18</sup> in their cases of splenic neutropenia. The changes observed in the megakaryocytes rather closely resembled those described by Dameshek<sup>11</sup> and others in essential thrombocytopenic purpura. Both pictures have been seen to revert to normal following splenectomy. To be sure, in all the reported cases of splenic neutropenia there has been splenomegaly; but it is well known that in a large percentage of cases of essential thrombocytopenia there is no splenomegaly, and that in some there is some degree of neutropenia. It seemed not unlikely that a similar sort of splenic dysfunction

might cause severe neutropenia such as in this case, without enlargement of the spleen.

The disappointing postoperative course has proven these early expectations to be erroneous. There was no remission of the neutropenia, and while there was a remarkable immediate proliferation of platelets by the megakaryocytes, the thrombocytosis was of short duration. It now appears evident that the spleen was not responsible for the neutropenia in this case, and that the primary defect, perhaps in the marrow, perhaps in some other organ which influences the marrow, has not been influenced by splenectomy. What the basis for this defect of maturation may have been remains undetermined.

It is evident, then, that not all cases of idiopathic neutropenia with cellular marrow are benefited by splenectomy. Two siblings with a similar severe neutropenia, and without splenomegaly, have in recent years been observed in this clinic, and will eventually be reported in detail. The marrow of one was examined, and showed a failure of maturation of the neutrophils very similar to that in the present case. This child failed to benefit from medical therapy, or from splenectomy, and has since died of an intercurrent infection. In such cases there is presumably an inborn fault in the hematopoietic system, unrelated to exogenous toxins or to the influence of the spleen. The long history of symptoms in the present case suggests that it may be one of this group. Since these cases were not benefited by splenectomy, it would appear that in the absence of splenomegaly one should hesitate to treat neutropenia operatively.

#### SUMMARY

A case of severe chronic neutropenia without splenomegaly is reported. The disorder was characterized by chronic fatigue and many pyogenic infections, and by a persistently low neutrophil count. The marrow was cellular, with a defect in maturation in both granulocytic and megakaryocytic series. There was no improvement on the usual types of medical therapy, and splenectomy, while it was followed by transient thrombocytosis, likewise failed to induce a remission.

It is suggested that in the consideration of therapy for severe neutropenia, a lack of splenomegaly should be considered a contraindication to splenectomy.

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# STUDIES ON THE PHYSIOLOGY OF THE WHITE BLOOD CELL

## THE GLYCOGEN CONTENT OF LEUKOCYTES IN LEUKEMIA AND POLYCYTHEMIA\*

By RICHARD WAGNER, M.D.

IN A previous paper<sup>1</sup> a procedure has been described for determining glycogen in whole blood and isolated white blood cells. By this method, the average of the glycogen content of normal human blood is 5.5 mg. per cent. The average of the glycogen content per million normal total white blood cells is 2.54 γ. Plasma, red blood cells, and blood platelets do not contain any measurable amounts of this carbohydrate.

In the present investigation this method has been applied to the study of the glycogen content of whole blood and white blood cells in cases of leukemia and polycythemia. Cases of leukemia with only one predominant cell form are particularly suitable for studying the biology of the white blood cells as far as their glycogen metabolism is concerned. Polycythemia was included in this study because of its possible relation to leukemia and the large amounts of glycogen detected in whole blood as well as in isolated leukocytes in this disease. Some experiments dealing with the glycogen content of rabbit leukocytes are also included in this study.

### TECHNIC

The glycogen determinations in whole blood are carried out on 1 cc. samples according to a micro modification of the Pflüger<sup>2</sup> method. The material for the glycogen determinations in white blood cells is collected in a Cushman<sup>3</sup> tube, analytically weighed, and its glycogen content determined according to the same method as that of whole blood. For the quantitative evaluation of the results a white blood cell count is done on the cell layer, using a white blood cell pipet, and the volume of its first subdivision is determined by calibration with mercury. The dilution for the cell count is 1 to 100. Both the weight of the total white blood cell layer and the weight of its amount in the blood pipet are determined.† The amount of glycogen per million wet white blood cells is then calculated according to the following formula:

$$\text{Glycogen per million white blood cells} = \frac{A \times B}{C \times D \times E}$$

A—Glycogen found in the white blood cell layer (γ).

B—Weight of the white blood cell layer used for the cell count (γ).

C—Volume of the first subdivision of the blood pipet (cmm.).

D—Number of white blood cells in the layer (millions per cmm.).

E—Weight of the white blood cell layer (γ).

### RESULTS

White blood cell layers collected in the described manner cannot be considered as homogeneous. They consist of myeloid and lymphoid cells. There is some difficulty

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†This rather complicated procedure is the least time-consuming method for dealing with living white blood cells without losing some of the glycogen as a result of the intense enzymatic activity of the leukocytes.

in evaluating the glycogen content of a white blood cell layer as long as the content of its separate constituents is unknown. In this respect studies on leukemia with one predominant cell type are helpful.

TABLE 1.—*Glycogen Content of Whole Blood and White Blood Cells in Chronic Myelogenous Leukemia*

Exper. Number	W. B. C. per cmm.	Blast Cells	Pro-my-elec.	My-elec.	Meta-my-elec.	Polys.	Eo.	Baso.	Mono.	Lympho.	Plasma Cells	Glycogen in Whole Blood Determined	Glycogen per Million W. B. C. Determined	Glycogen Content of W. B. C. Calculated
		%	%	%	%	%	%	%	%	%	%	mg. per cent	γ	per cent
182	9,450	—	—	8	18	64(23)*	1	—	2	7	—	6.0	45.50	6.10
158†	10,100	—	—	—	—	45(9)	1	2	32	20	—	6.1	4.16	0.64
84	24,700	—	—	2	9	79(65)	—	2	2	6	—	12.3	3.81	0.51
103	38,250	—	—	2	3	73(27)	4	1	1	16	—	31.5	4.26	0.63
192	38,500	3	3	2	21	54(13)	4	1	2	9	1	19.4	4.70	0.67
16	43,400	4	—	16	—	57(10)	—	19	3	1	—	24.9	3.88	0.51
114	50,000	1	—	19	38	34	—	1	3	4	—	17.6	3.16	0.41
115	60,000	19	12	21	8	32(16)	1	6	—	1	—	37.0	3.87	0.60
185	60,000	—	10	11	10	57(12)	—	3	3	6	—	16.4	2.43	0.32
176	145,300	1	3	13	21	51(20)	2	1	1	7	—	22.9	2.23	0.30
180	355,000	2	7	34	27	27(15)	1	1	—	1	—	48.2	1.98	0.25
179	440,000	1	4	31	28	32(17)	1	—	1	2	—	97.0	1.37	0.17
149	576,000	5	8	43	22	16	2	1	1	2	—	>114.0	2.68	0.36

\* The figures in parentheses indicate band forms.

† The figures in this column are calculated on the assumption that the nitrogen content and water content in human leukocytes are the same as in those of rabbits (cf. experiment 198). The figures are calculated by dividing the glycogen content per million W. B. C. by 8.2.

‡ Diagnosis "chronic myelogenous leukemia" confirmed by bone marrow puncture.

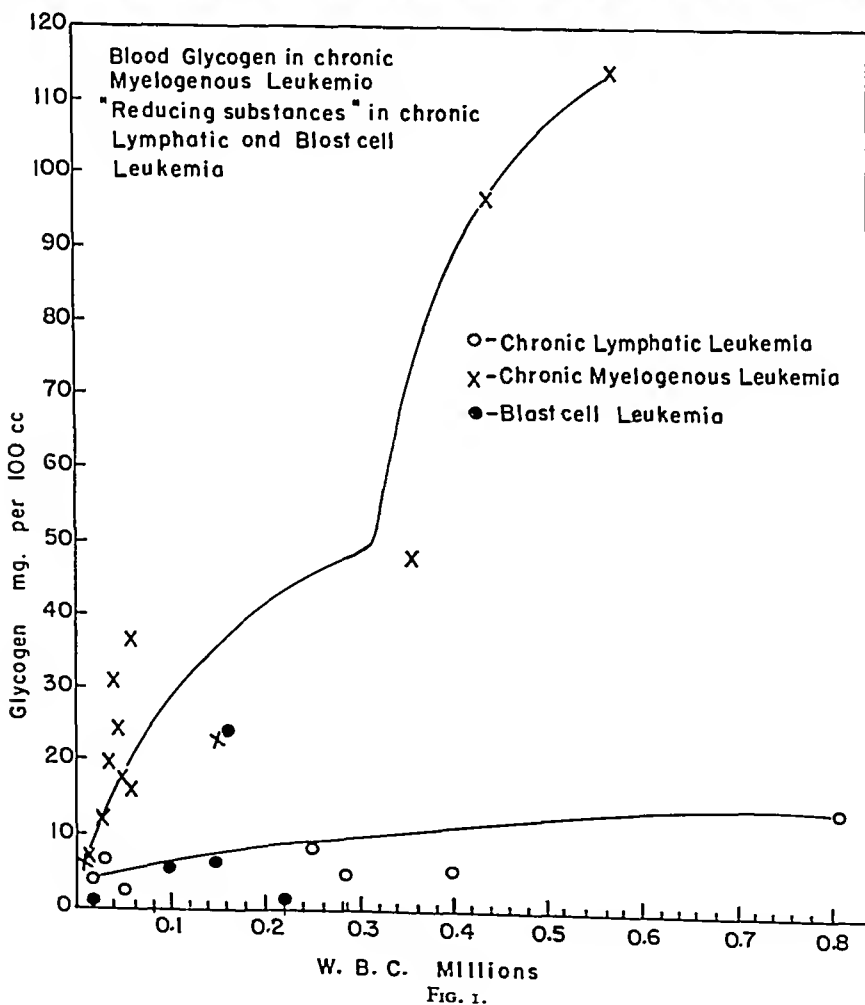
TABLE 2.—*"Reducing Substances" of Whole Blood and White Blood Cells in Chronic Lymphatic Leukemia*

Exper. Number	W. B. C. per cmm.	Blast Cells	Polys.	Eo.	Mono.	Lympho.	Reducing Substances in the Alcohol Precipitates Following Acid Hydrolysis	
							In Whole Blood Determined	Per Million W. B. C. Determined
							mg. per cent	γ
157	15,000	—	32(2)	1	8	59	4.2	0.12
169	30,000	—	15	3	—	82	6.7	0.75
177	48,700	—	17(4)	—	8	75	2.9	0.46
184	245,000	1	3(2)	—	—	96	8.7	0.24
175	287,280	—	2	—	—	98	4.9	0.07
130	403,000	—	—	—	—	100	10.9	0.37
161	810,000	—	—	—	—	100	14.4	0.22

1. *Glycogen Content of Whole Blood and Isolated White Blood Cells (per Million) in Leukemia.* Tables 1, 2, and 3 contain the experimental data on 25 cases of different types of leukemia. The graphic picture (fig. 1) reveals the relation between the number of white blood cells per cmm. and the concentration of glycogen and

TABLE 3.—"Reducing Substances" of Whole Blood and White Blood Cells in Acute (Blast Cell) Leukemia

Exper. Number	W. B. C. per cmm.	Blast Cells		Pro-myeloc.	Mye-loc.	Meta-myeloc.	Polys.	Eo.	Baso.	Mono.	Lym-pho.	Reducing Substances in the Alcohol Precipitates Following Acid Hydrolysis	
		Myelo. blasts	Lym-pho. blasts									In Whole Blood Determined	Per Million W. B. C. Determined
		%	%	%	%	%	%	%	%	%	%	mg. per cent	γ
154	103,000	—	95	—	1	—	—	—	—	—	4	5.2	0.10
92	220,000	—	93	—	—	—	2	—	—	—	5	1.1	0.08
191	17,200	—	95	—	—	—	3	—	—	2	—	<1.0	0.19
159	149,000	96	—	—	—	—	1(1)	—	—	2	1	6.2	0.30
188	158,400	89	—	—	2	4	5(3)	—	—	—	—	24.5	0.80



"reducing substances"\* respectively in mg. per cent for this group. There is practically no increase of "reducing substances" in chronic lymphatic leukemia with increasing number of white blood cells per cmm. In spite of a count of 810,000 W. B. C. per cmm. the "reducing substances" are about on the same level as in normal blood. On the other hand, in chronic myelogenous leukemia there is a considerable increase in almost linear proportionality with the number of cells. The highest value ever observed was found in a case of chronic myelogenous leukemia with 576,000 W. B. C. per cmm. and 43 per cent myelocytes (experiment 149). In 5 cases of acute leukemia, myeloblastic as well as lymphoblastic, the "reducing substances" are again low, in experiments 92 and 191 almost negligible. Only in experiment 188 is the obtained value higher than that in the other acute cases. However, this is the only case of this group where the percentage of more mature elements is relatively higher than in the other 4 cases.

From figure 1 the conclusion is reached that it is evidently the total number of *granulated* leukocytes which determines the polysaccharid content of whole blood. Since in previous studies<sup>1</sup> the blood plasma, the red blood cells and blood platelets were found to be free from glycogen, it was to be expected that glycogen determinations on isolated leukocytes might help to explain the differences of the glycogen content of whole blood in the different types of leukemia. In tables 1, 2, and 3 the concentration of glycogen and "reducing substances" respectively per million W. B. C. is recorded.

In all cases of chronic lymphatic leukemia the content of "reducing substances" per million W. B. C. is below 1  $\gamma$  (average 0.32  $\gamma$ ). In experiment 175 the lowest value of all observations on white blood cells was found. In all instances of blast cell leukemia the value per million W. B. C. is likewise below 1  $\gamma$  (average 0.29  $\gamma$ ), approximately within the same range as in lymphatic leukemia. In all cases of chronic myelogenous leukemia the glycogen content is above 1  $\gamma$  (average 3.21  $\gamma$ ; experiment 182 excluded).

The conditions under which experiment 182 was carried out may explain the strikingly high value of 45.5  $\gamma$ . The analysis in whole blood as well as in W. B. C. was done immediately after x-ray treatment. The number of W. B. C. dropped from 374,000 to 9450 cells per cmm. The whole blood glycogen determination was carried out on blood collected from the hematocrit tube after the cells had been resuspended. Some of the glycogen might have been destroyed by this manipulation. The high glycogen content per million W. B. C. may be interpreted as evidence of the capacity of white blood cells to phagocytize glycogen freed from leukocytes following x-ray treatment.

2. *Glycogen Content of Granulated White Blood Cells in the Rabbit (Weight Percentage).* In all of the preceding experiments only the glycogen content per million W. B. C. was determined. Since the weight of the individual white blood cell is not known, it is not possible to come to a conclusion as to the weight percentage of glycogen

\*The term "reducing substances" refers to the reducing substances in the hydrolyzates of the alcohol precipitates which are not considered as wholly composed of true glycogen.

Plasma cell leukemia is not included in this investigation, because only 1 case has been studied. However, the concentration of "reducing substances" per million W. B. C. was strikingly high in this instance.

†Assuming 60 per cent granulated leukocytes in normal blood, the average of the glycogen content per million normal granulated W. B. C. is 4.23  $\gamma$ .

in white blood cells. This would be of interest for comparison with the glycogen content of other tissues, such as liver or muscle. However, this problem can be approached by two kinds of experiments: (a) Granulated W. B. C. of uniform character can be collected from sterile exudates experimentally produced in animals. Glycogen and total nitrogen can be easily determined in the same suspension of leukocytes, since there is no disturbing admixture of platelets present. This procedure is superior to the study of pus cells, since in pus cells the enzymatic activity and its destructive influence on the glycogen content are uncontrollable. Pus cells and living W. B. C. are biologically different as to their glycogen metabolism. (b) Another way of proceeding is to collect white blood cells from normal individuals by the regular procedure in the Cushman tube. One portion of the cells can be analyzed for glycogen without further separation from the remainder of plasma and blood platelets. In another portion, which has to be entirely purified from blood platelets and plasma, the total nitrogen is determined. However, the latter

TABLE 4.—*Glycogen Content of Whole Blood and White Blood Cells in Polycythemia*

Exper. Number	W. B. C. per cmm.	Polymorphonuclear W. B. C.	Glycogen in Whole Blood	Glycogen per Million W. B. C.	Glycogen per Million Polymorphonuclear W. B. C.	Glycogen in Wet Granulated W. B. C. [Glycogen per Million] 8.2
			Determined	Determined	Calculated	Calculated
		%	mg. per cent	$\gamma$	$\gamma$	%
14t	16,600	81	22.4	5.62	6.94	0.85
165	18,000	85(9)	33.4	1.62	1.94	0.24
183	18,850	74	14.3	6.10	8.24	1.00
189	9,500	58	24.3	7.8	13.40	1.64

method offers certain technical difficulties, first of all the necessity of purifying procedures which are indispensable for a correct determination of nitrogen. Therefore the first procedure was chosen with the following results.

*Experiment No. 198.* The procedure of collecting white blood cells followed the technic of De Haan.<sup>4</sup> A rabbit (not fasting) was injected intraperitoneally with 300 cc. of a physiological saline solution. Twenty-four hours later another 150 cc. were injected; after 1.5 hours the fluid was withdrawn. It represented a homogenous suspension of granulated leukocytes, and this was verified microscopically. In order to prevent clotting the white blood cell suspension was immediately mixed with 0.6 per cent sodium citrate in physiological saline solution. The suspension was then centrifuged on the angle centrifuge at high speed, the cells resuspended in the sodium citrate-sodium chloride solution and made up with it to 5 cc. in a volumetric flask. The suspension contained 3050 cells per cmm.

In 3 cc. of the white blood cell suspension (9,150,000 W. B. C.) 92.5  $\gamma$  glycogen and in 1.5 cc. (4,575,000 W. B. C.) 120  $\gamma$  nitrogen were found; this is 10.1  $\gamma$  glycogen and 26.2  $\gamma$  nitrogen or 164  $\gamma$  protein per million W. B. C. Assuming a water content of 80 per cent, granulated leukocytes of rabbits contain 1.23 per cent glycogen..

3. *Influence of Intravenous Administration of Glycogen on the Glycogen Content of Granulated White Blood Cells in the Rabbit.* *Experiment 196:* After intravenous injection of 3 grams of glycogen "Pfanstiehl" C.P. (15 cc. of a 20 per cent solution) in a rabbit, following the technic of Morris,<sup>5</sup> the glycogen content of the isolated

white cells increased from 1.36  $\gamma$  per million (0.17 per cent) in fasting conditions before the injection to 7.9  $\gamma$  per million (0.96 per cent) 30 minutes after the injection.

4. *Glycogen Content of Whole Blood and Isolated White Blood Cells (per Million) in Polycythemia.* In table 4 the experimental material on 4 patients with polycythemia is shown. The glycogen content of the whole blood not only exceeds the normal standards by a considerable amount, but the glycogen content per million W. B. C. is extremely high in 3 of the cases examined, compared with that of normal individuals. Only in glycogen storage disease was a higher glycogen content per million W. B. C. encountered.

#### DISCUSSION

There is a wide divergence of opinion as to whether or not all of the reducing substances in the alcohol precipitates of blood following acid hydrolysis originate from glycogen. It was shown that the white blood cell is the main carrier of reducing substances of polysaccharid character in whole blood.<sup>1</sup> The studies on leukemic blood give a more precise answer as to which type of white blood cell group contains substances with the properties of glycogen. From the following facts it is evident that at least part of the polysaccharids present in blood and its constituents is glycogen.

1. The general chemical properties of these carbohydrates are the same as those of liver or muscle glycogen. They are precipitable by alcohol and can be hydrolyzed with acids. The breakdown product is fermentable by yeast. They can be extracted with water and determined in the aqueous solution with sufficient exactness if present in large enough amounts, as for instance in chronic myelogenous leukemia.

2. Bridge and Holt<sup>6</sup> identified the polysaccharid isolated from blood in glycogen storage disease as glycogen by its chemical properties, comparing it with a control glycogen which had been reprecipitated from rabbit liver. The small variations from the control were quantitative and not qualitative.

Further proof for the glycogen character of the polysaccharid in blood is its enzymatic breakdown in isolated white blood cells. It shows the same rate of disappearance known to occur in other organs with active glycogen metabolism such as the liver.<sup>1</sup>

In a recent study Verheugt<sup>7</sup> expressed the opinion that in blood of normal men no glycogen is present, at least not in quantities that can be measured by Pflüger's method. Our present studies leave no doubt that the polysaccharid found in granulated white blood cells is true glycogen. The linear increase of "reducing substances" in whole blood of chronic myelogenous leukemia with increasing number of white blood cells may be considered as satisfactory evidence for the exclusive presence of glycogen in the granulated leukocytes. The lack of increase in chronic lymphatic leukemia on the other hand rules out the presence of any measurable amount of this carbohydrate in the lymphocytes. The same holds true for blast cells. The studies on isolated white blood cells of one uniform type are consistent with the above assumption. *The "reducing substances" found in lymphocytes and blast cells are not glycogen.*

The exclusive presence of glycogen in the granulated white blood cell explains certain technical peculiarities of the determinations in blood and its constituents. The results of duplicate determinations in normal whole blood often show considerable discrepancies, while determinations in isolated leukocytes show good agreement. There are reducing substances in whole blood which interfere with the exact determination of glycogen. It was shown in previous studies<sup>1</sup> that the ribonucleic acid content of blood platelets explains at least a large part of the reduction obtained after acid hydrolysis of the alcohol precipitates of whole blood. All determinations of whole blood glycogen without yeast fermentation resulted in erroneously high values. In the white blood cell the glycogen is concentrated and the interference of other reducing substances is negligible.

From these investigations on leukemia can be drawn important conclusions as to the physiology of the white blood cells. It is interesting that during the development of the granulated polymorphonuclear leukocyte glycogen appears in considerable amounts as a cell constituent in the myelocytic phase, while the blast forms are still free from glycogen. It might be concluded that the content of a reserve carbohydrate in the granulated white blood cell increases with increasing maturity.\* The amount is probably determined by the increasing phagocytic and ameboid activity of the maturing cell. The lymphocytes, on the other hand, representing a biologically different cell type with a different function, are free of glycogen.

In the experiment on the peritoneal exudate of rabbits (experiment 198) it could be shown that the glycogen content of the granulated white blood cells is in the same order of magnitude as that of the striated muscle. With regard to its physiological peculiarities, the tissue of the granulated white blood cells has at least one function in common with the striated muscle—that is, the motor activity. As in the case of the muscle there can likewise be demonstrated a wide range of the glycogen content for the granulated white blood cell.

In human muscle, for instance, the average glycogen content amounts to 0.4 per cent (Moscatti<sup>8</sup>). In the muscles of dog a maximum of 3.72 per cent was determined (Schöndorff<sup>9</sup>). Exercise and intake of food exert great influence upon the glycogen content of the musculature. In fasting animals it is 0.1 to 0.4 per cent, after intake of food 0.7 to 1.0 per cent (Böhm<sup>10</sup>).

For comparison an attempt was made at computing the weight percentage of glycogen in human wet white blood cells from the glycogen content per million, assuming the same nitrogen and water content in the human cells as in those of rabbits (cf. last column of tables 1 and 4). Excluding experiment 182, in 7 instances the glycogen content was found to be between 0.42 and 0.67 per cent. In the other 5 instances it was below this value. It may be significant that in the cases with the highest W. B. C. counts the lowest glycogen contents were encountered.

\* The appearance of myelocytes in the peripheral blood seems to be of decisive influence on the glycogen content of whole blood (cf. experiments 149 and 176). The highest glycogen value was observed in experiment 149 in the presence of 248,000 myelocytes in contrast to the relatively low value in experiment 176 with only 21,890 myelocytes. The figures for the mature polymorphonuclear leukocytes (92,600 and 74,100) are very close in the two experiments.

While the glycogen content of leukocytes collected from patients with chronic myelogenous leukemia was found to be within the same order of magnitude as that of normal individuals, some variation of this value could be demonstrated under experimental conditions as well as in disease. The increase after intravenous injection of glycogen in the rabbit is evidently the result of phagocytosis and storage (experiment 196). Another example of phagocytosis was previously mentioned. In experiment 182 (table 1) an extremely high glycogen concentration of 45.5  $\gamma$  per million W. B. C. was encountered following x-ray treatment. In glycogen storage disease we found up to 25  $\gamma$  per million granulated cells (3.05 per cent). Bridge and Holt<sup>6</sup> calculated for leukocytes in glycogen storage disease a glycogen concentration of from 6 to 10 per cent.

More difficult to interpret are the high glycogen values of leukocytes which we found in polycythemia. They cannot simply be explained by phagocytosis and storage. Other factors may come into play. In view of the intensive enzymatic breakdown of glycogen in leukocytes<sup>1</sup> it is probable that an active synthesis of this carbohydrate likewise takes place within the white blood cell. Willstätter and Rhodewald's<sup>11</sup> experiments on the enzymes of leukocytes may be considered as evidence of such a synthesis. They deny the occurrence of direct glycolysis of glucose in blood and explain the disappearance of blood sugar as the result of glycogen synthesis and glycogenolysis. The granulated leukocyte is equipped with the specific capacity of storing energy in the form of glycogen. It is easy to conceive of glycogen as a reserve carbohydrate, being present in blood in the granulated leukocytes, while its breakdown product glucose must be readily available and can promptly be transported to the tissues of the body wherever there is some immediate need for it. In this way a new function is attributed to the granulated leukocyte in the system of tissues serving the carbohydrate metabolism.

The study of the glycogen concentration in isolated living leukocytes may have further implications as to the physiology and pathology of this cell group and their enzymatic activity. In leukemia it is easy to collect large amounts of material of one uniform cell type. Other cell constituents, such as lactic acid, can be likewise studied by using the same procedure of isolation and quantitative evaluation. Particularly glycolysis ought to be investigated on isolated leukocytes of leukemic blood.

#### SUMMARY AND CONCLUSIONS

The technic of determining glycogen in isolated white blood cells was applied to the study of the different types of leukemia and of polycythemia, in order to obtain information on the physiology of the white blood cell. From this study it is concluded that the granulated leukocyte is the only carrier of glycogen in whole blood. The "reducing substances" in lymphocytes and blast cells are not considered as true glycogen.

The glycogen content of wet white blood cells in the rabbit amounts to about 1 per cent. In the human being a range of from 0.17 to 0.67 per cent was calculated. In disease higher percentages occur, in polycythemia up to 1.64 per cent and in glycogen storage disease up to 3.05 per cent.

The glycogen concentration of normal white blood cells is within the same range as that of the striated muscle.

I acknowledge with gratitude my indebtedness to Dr. William Dameshek for giving me the opportunity of analyzing the blood of some of the patients studied. Miss M. H. Campbell, Miss H. A. Clark, and Miss L. M. Garofalo have aided in carrying out many of the blood counts.

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# OBSERVATIONS ON THE ANEMIA IN DUCKS INFECTED WITH *P. LOPHURAE*\*

By R. H. RIGDON, M.D. AND H. H. ROSTORFER, Ph.D

THE studies of many investigators have emphasized the significance of the anemia in malaria.<sup>1-4</sup> The degree of anemia in the duck infected with *P. lophurae* is proportional to the number of parasites in the peripheral blood. In most cases of *P. lophurae* infection in ducks a marked drop in the total number of red cells occurs as the peak of parasitemia is approached; the number of red cells and the level of hemoglobin return to normal within 3 to 5 days following the peak of infection.<sup>4</sup> Hill<sup>5</sup> concluded from her studies on pigeons infected with *P. relictum* that death results from the anemia. Rigdon and Varnadoe<sup>6</sup> have recently shown that life may be prolonged in ducks infected with *P. lophurae* by the frequent injection of normal duck blood.

Apparently few observations have been made on the erythrocytes in the duck and the changes they may show in a severe anemia. Hewitt has contributed many hematological observations on both the normal and malarial infected ducks.<sup>4,7</sup> He has emphasized the occurrence of an anemia and the fall in hemoglobin in *P. lophurae* infected birds. Hewitt has called attention to the varying degrees of polychromemia in the red cells; the fact that the nuclei are larger than those of mature red cells; and also, that they may be round rather than elliptical. Binucleated red cells, anucleated forms, and deeply basophilic erythroblasts may be found in the peripheral blood during severe infections.

Taliaferro and Kluver<sup>3</sup> have reviewed the subject and supplemented our knowledge of the hematology of malaria in Panamanian monkeys. They emphasized the occurrence of anemia and a decrease in the amount of hemoglobin. Normoblasts and anisocytosis were found in the peripheral blood during malarial infections in monkeys.

The physiological studies made in this laboratory on the oxygen-carrying capacity of the blood in experimental malaria and the effect of high altitudes on the course of *P. lophurae* infection in ducks have caused us to study further the changes which may occur in the duck's erythrocytes in malaria.<sup>8-10</sup>

## METHODS AND MATERIALS

White Pekin ducks, 2 to 4 weeks of age, infected with *P. lophurae* were used for this study. Blood for smears was obtained by puncturing a vein in the legs. They were stained with a combination of Giemsa's and Wright's stains. The parasitemia was determined by counting the number of parasitized cells per 500 red cells. The number of both the young and adult red cells also was determined per 500 red cells.

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The erythroblast was differentiated from the erythrocytes in this study by the bluish staining of the cytoplasm and by the spherical shape of the nucleus and cell body in comparison to the more elliptical shape of the mature red cells.

The same blood smears used for determining the degree of parasitemia were used to measure the size of the different red cells. Twenty-five to 100 cells were measured each day during the infection to establish their average size. Measurements were made with an ocular micrometer. The maximum and minimum lengths of both the cell and the nucleus were recorded. Standard technics were used for counting the erythrocytes. Hayem's fluid was used for the diluent.

Blood for the determination of hemoglobin, hematocrit, color index, and cell volume was obtained by cardiac puncture from two to three birds and pooled. Liquaemin\* in a dilution of one to nine was used as the anticoagulant. The functional hemoglobin was calculated from the oxygen capacity, which was determined by equilibrating the blood with air at 38° C. and analyzing it on the Van Slyke manometric apparatus. The percentage of functional hemoglobin was obtained by allowing the colorimetric hemoglobin to represent 100 per cent and considering the hemoglobin determined from the oxygen capacity as a percentage of the colorimetric hemoglobin. The latter was determined by the method of Schultze and Elvehjem.<sup>11</sup> The color index was calculated by dividing the hemoglobin in grams obtained from the oxygen capacity, by the erythrocyte count. The hematocrit was obtained by centrifuging 10 cubic centimeters of blood at 3000 r.p.m. The cell volume was obtained by dividing the volume of cells in 10 cubic centimeters of blood by the erythrocyte count. Smears were prepared from the femur bone marrow, the spleen, and the liver from malarial infected and control birds. A small portion of the tissue was placed in a drop of duck plasma on a glass slide and carefully teased out. Smears were made from this preparation and were stained similarly to the blood.

#### EXPERIMENTAL

The degree of parasitemia and the severity of the accompanying anemia varied with the age of the duck and the number of parasites injected. The course of a typical malarial infection in a fatal case is shown in figure 1. The degree of the anemia usually is proportional to the parasitemia. Accompanying this anemia there is a progressive increase in the number of erythroblasts in the peripheral blood. In these ducks the peak of the parasitemia is reached on the fifth day and then the number of parasites rapidly decreases until only a few are present on the eighth day (fig. 2). The most severe anemia is present approximately 24 hours following the peak of the parasitemia. In the ducks that survive the total number of red cells rapidly increases and reaches approximately normal levels by the tenth day following inoculation. The level of hemoglobin, the color index, and the hematocrit all decrease at a parallel rate with the decrease in the total number of erythrocytes; also, they increase correspondingly with the increase in the number of red blood cells in the peripheral circulation (fig. 2).

\* Supplied through the courtesy of Roche-Organon, Inc., Nutley, N. J.

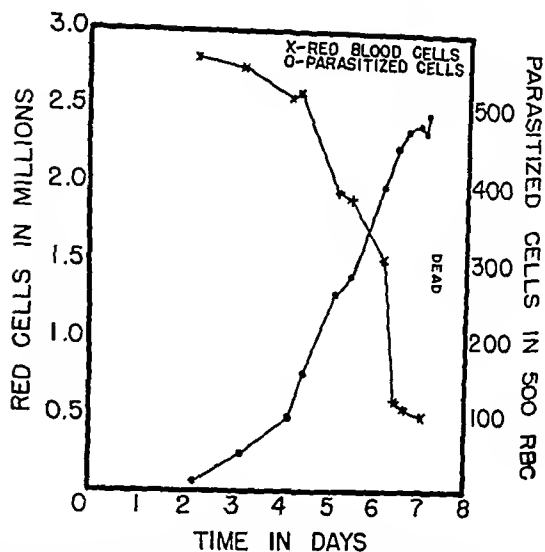


FIG. 1. This represents the normal development of the parasitemia and the anemia in young infected with *P. lophurae*. Usually there occurs a decrease in the degree of the parasitemia preceding time of death.

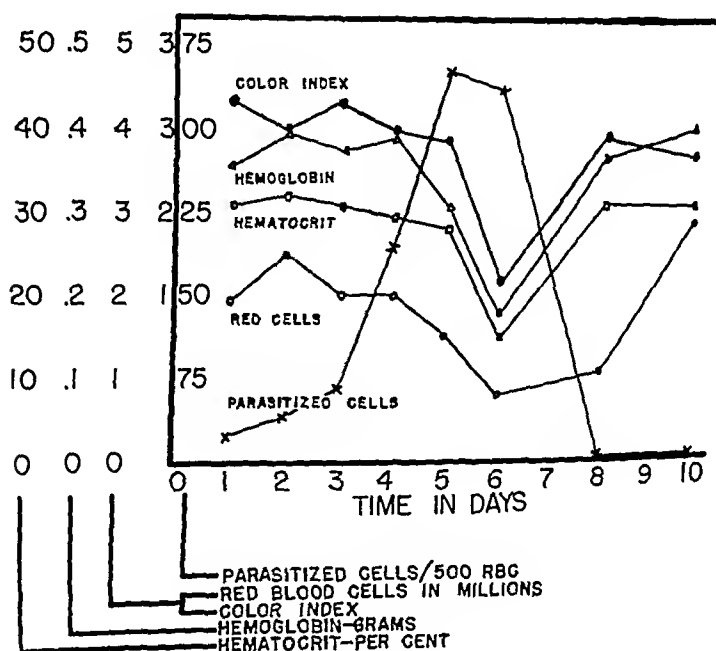


FIG. 2. Accompanying the anemia in *P. lophurae* infections in ducks there is a decrease in cell volume, hemoglobin, and hematocrit. With a return in the number of erythrocytes to normal there is a corresponding increase in the cell volume, hemoglobin and hematocrit.

The proportion of young red cells to adult erythrocytes during the infection is indicated by the graph in figure 3. There is a variation in the size and shape of the

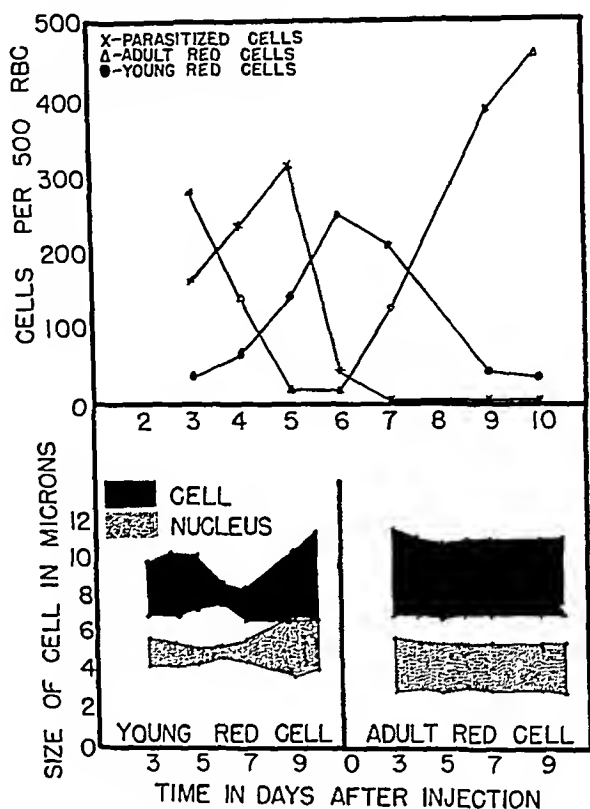


FIG. 3. The adult and young red cells and their nuclei are measured in both their maximal and minimal diameters and the average is plotted on the lower portion of this figure. The areas between the maximum and minimum measurements of both the cells and their nuclei are indicated on the diagram. The adult erythrocyte with its nucleus maintains a uniform size and shape during the course of the infection, while the young erythrocyte with its nucleus varies both in size and shape during this time. At the time of the greatest number of young cells in the peripheral blood many of the young erythrocytes and their nuclei are small and almost spherical. With the subsequent decrease in the number of young cells the young erythrocytes increase in size and their shape approaches that of an elliptical body as indicated by the diagram in the lower half of this figure.

The figure in the upper half also indicates the rapid increase in the number of parasitized cells with a corresponding diminution in the number of adult erythrocytes that occurs until the peak is reached on the fifth day of the infection. There is an increase in the number of young erythrocytes until approximately 24 hours following the peak of the parasitemia, at which time the number of young cells rapidly decreases. This is accompanied by a corresponding increase in the number of adult types of erythrocytes in the peripheral blood.

erythroblasts in the peripheral blood during the course of the infection as shown by the diagrams in figure 3. Only typical adult erythrocytes are included in the group

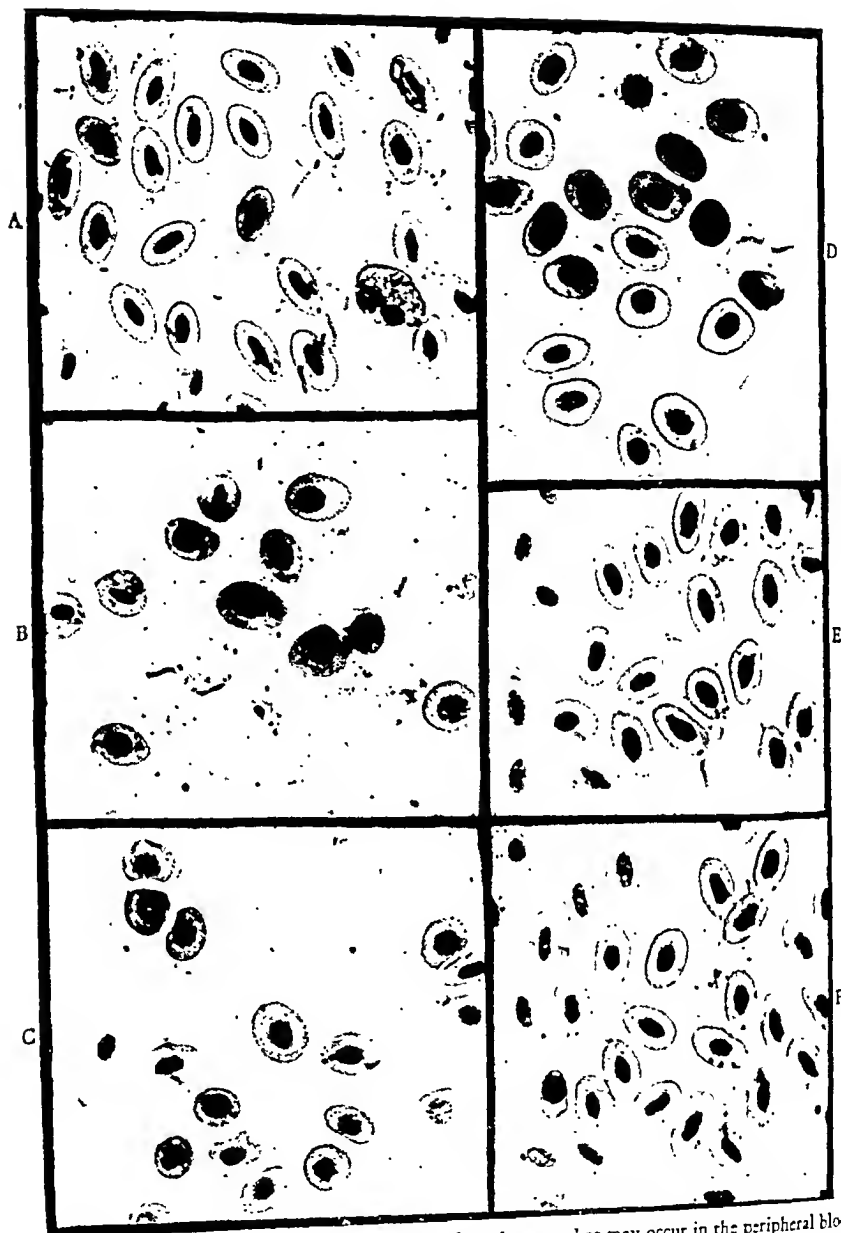


FIG. 4. This shows the variations in the type of erythrocyte that may occur in the peripheral blood of a duck that survives a severe malarial infection. *A*, normal erythrocytes as observed on the third day following inoculation; *B*, on the fifth day some of the erythrocytes are larger and others are smaller than a normal adult red cell. The cytoplasm stains a bluish purple color. *C*, few normal-appearing adult erythrocytes are present on the sixth day. Small round erythroblasts are conspicuous at this time. *D*, the peak of the parasitemia occurs on the fifth day. The cells on the seventh day are frequently large and some are elliptical in shape while others are spherical-like in shape in the stained smears. The cytoplasm usually stains a deeper blue than it does in adult erythrocytes. *E*, the erythrocytes on the ninth day are indistinguishable from normal red cells. *F*, the erythrocytes are normal on the tenth day following inoculation.

indicated as "Adult Red Cells" in figure 3. It is obvious from these data that this cell remains uniform in size and shape throughout the period of infection. In contrast to the "Adult Red Cell," the erythroblasts vary widely in both size and shape during the course of the infection as shown by the diagram in figure 3 indicated as "Young Red Cells." These young cells at first are elliptical in shape and slightly smaller than the adult erythrocyte; however, at the time of the greatest number of erythroblasts in the circulation many of these young cells are small and almost spherical in shape. These small round cells are found in significant number only in those ducks that have a very high degree of parasitemia, a severe anemia, and survive the infection. Two to three days following the peak of parasitemia the erythroblasts become larger and approach the shape of an elliptical body.

TABLE 1.—*Different Ages of Erythrocytes Observed in P. lophurae Infection in Ducks*

Duck No.	Exp. Day	Cells of different ages in the blood and hematopoietic tissue											
		Blood			Bone Marrow			Liver			Spleen		
		A*	B	C	A	B	C	A	B	C	A	B	C
Control	0	49	0†	1	207	614	37	0‡	19	0	12	4	70
1	5	16	120	18	19	134	204	19	100	54	17	106	91
2	5	—	—	—	90	139	442	38	81	37	43	44	118
3	5	9	100	38	1	30	122	—	—	—	—	—	—
4	5	13	101	31	67	383	272	40	100	71	25	100	59
5	5	15	104	24	14	45	569	87	136	171	60	100	69
347	6	81	110	6	13	100	49	60	103	10	25	78	101
354	6	159	43	2	23	114	33	—	—	—	—	—	—
350	6	145	114	4	34	234	233	51	108	8	42	100	114

\* A: Young red cells—illustrated in fig. 5B.

B: Middle-aged red cells—illustrated in fig. 5C.

C: Youngest type of red cells—illustrated in fig. 5D.

† Counted 357 normal adult erythrocytes in peripheral blood.

‡ Counted 100 hepatic cells in this smear.

Since no satisfactory grouping could be made for measuring the young red cells within the peripheral blood, typical cells were photographed at different intervals during the course of the infection to show the variations in their size and shape (fig. 4). It is obvious from these photographs that the shape of a young erythroblast in a stained smear approaches that of a sphere. Furthermore, many of the cells are small on the fifth and sixth days of the infection at a time when the greatest strain is put upon the hematopoietic system. Erythrocytes in the peripheral blood, bone marrow, spleen, and liver are classified as to age and the results are given in table 1. All ducks with malaria show a marked increase in the percentage of young cells of the erythrocytic series. Near the time of death many of the immature forms of erythrocytes enter the peripheral circulation. The hematopoietic responses of the femur bone marrow, spleen, and liver are similar in that each shows a hyperplasia of the erythroblastic tissue. Apparently the greatest number of red cells are formed in the bone marrow in comparison with the liver and the spleen.

The tissue from 5 ducks was taken immediately following death from malaria and that from 3 ducks the day following the time of the peak of the infection. In each of these 8 ducks the bone marrow, liver, and spleen showed a marked increase in the number of young erythrocytes when compared with the control ducks of similar age (table 1). The bone-marrow of some of the ducks showed a predom-



FIG. 5. The cells in *A* are normal adult erythrocytes. The cells in *B* are "young erythrocytes." Note that their shape is neither a perfect elliptical body nor a sphere. The cells in *C* are classified as "middle-aged erythrocytes." Note the large nucleus and the relatively small amount of cytoplasm. A majority of the cells here show clear areas within the cytoplasm. One large cell is present here which shows only a little cytoplasm. This is one of the youngest forms of the erythroblasts. Few of these cells ever get into the circulation. The cells in *D* are the youngest types of erythrocytes. Note the cells in mitosis. The youngest forms of the erythroblasts are seldom found in the peripheral blood.

ance of "very young erythroblasts." These cells are large and round (fig. 5D). The nucleus fills the greater portion of the cell. The chromatin of the nucleus is fine and loosely packed. The cytoplasm is represented by a narrow band of a blue staining material. The larger of these "very young erythroblasts" are approximately 12-15 microns in diameter. The cell classified in this study as a "middle-aged erythroblast" is a round cell, smaller than the so-called "very young erythroblast" de-

scribed above. The nucleus is round and the particles of chromatin are larger and more compact than they are in the younger type of cell. There is also a larger amount of cytoplasm in these cells than in the younger cells. The cytoplasm stains a bluish purple (fig. 5C). In the cytoplasm of many of these cells there are large, clear unstained areas. Frequently the cytoplasm is almost completely replaced by these unstained areas (fig. 5C). The oldest type of young red cells present in the hematopoietic tissue is an elliptical-shaped cell with a similarly shaped nucleus. The cytoplasm stains bluish pink. The chromatin of the nucleus is coarse and more compact than it is in the younger cells (fig. 5B). In some of the ducks the bone marrow, liver, and spleen show a predominance of so-called "very young erythroblasts" while others show a majority of the "middle-aged type of erythroblasts." The cells in the peripheral blood of these ducks with malaria are primarily like those described above as "young red cells." Occasionally, however, few of the younger types of erythroblasts are present in the peripheral blood. Ducks dying from the disease may have a large number of the youngest forms of erythrocytes in the peripheral blood.

The youngest types of erythroblasts do not have parasites within their cytoplasm and only a few of the more mature erythroblasts have parasites within their cytoplasm. The absence of malarial parasites within these young cells is most conspicuous especially when these cells are present in the peripheral circulation.

#### DISCUSSION

The results of the observations made in this study on the development of the anemia and the determination of the amount of hemoglobin in ducks infected with *P. lophurae* are essentially the same as those reported by Hewitt.<sup>4</sup> The bizarre forms of erythrocytes that may be observed in the peripheral circulation emphasize the fact that pathological cells may develop in the blood-forming tissue and escape into the circulation. The presence of a large number of erythroblasts in the circulating blood which are more spherical than elliptical in shape indicates the morphological forms through which a normal erythroblast of the duck must pass to reach maturity. Smears from the bone marrow confirm the opinion that a young red cell is round and subsequently becomes elliptical in shape.

Young red cells in malaria usually are spherical when they enter the blood stream and apparently become elliptical as they grow older. This observation would indicate that there is no fundamental difference in the cell structure of these young cells which are produced in excessive numbers in malaria and the young cells that reach the peripheral blood under normal conditions. This change in the development of erythrocytes from a spherical form in the hematopoietic tissues to an elliptical-shaped cell may be the result of a variation in the environment. Hamburger<sup>12</sup> observed that erythrocytes of the horse and dog decreased in diameter as the plasma was diluted with distilled water. He pointed out the fact that the cells so treated become globular. Haden<sup>13</sup> has called attention to many of the problems associated with any study on the variations in cell diameter and cell volume. Any study on the red cells in the duck infected with *P. lophurae* is difficult to follow since the hematopoietic response is so rapid and the interval is so short between

the time of appearance of abnormal numbers of atypical cells in the peripheral blood and either complete recovery or death from the infection.

Magath and Higgins<sup>14</sup> found the erythrocytes in normal tame mallard ducks to vary in size from 9.9 to 13.4 microns long (average 11.2) by 5.9 to 8.9 microns wide (average 6.7). The nuclei of these cells were 5.0 to 7.0 microns long by 1.5 to 2.5 microns wide. The normal-appearing erythrocytes in the peripheral blood of young white Pekin ducks infected with malaria are 11.3 by 7.1 microns and their nuclei 5.6 by 2.9 microns. The more mature erythrocytes in the peripheral circulation are elliptical in shape in ducks with a low parasitemia. Some of the erythroblasts, however, are smaller and almost spherical at the time the peak of parasitemia is reached in the highly parasitized birds. These small erythroblasts occur at a time when the greatest number of young cells are present in the circulation. With a diminution in the degree of the anemia the young erythroblasts again become larger and approach the shape of an elliptical body. Apparently when the hematopoietic system is forced to the point of maximum production the erythroblasts in the ducks may leave the hematopoietic centers as small round cells. Normoblasts are usually larger than erythrocytes when observed in the circulation in acute anemia in man. It is suggested that this difference in man and duck may be explained by the probability that life in man is not compatible with such an acute and severe strain on the hematopoietic system. In chronic anemia in man we have microcytes in the peripheral blood. These small round erythroblasts in ducks are found in significant numbers for 24 to 48 hours only in the few birds that survive a severe malarial infection.

It is of interest to observe that the small type of erythroblast is present in the circulation in the ducks in significant numbers for only a short time. The question arises: Do these small young cells develop into the larger type of erythroblasts and then subsequently become mature red cells? The rapidity of the recovery from the anemia might suggest that such occurs. These young cells are more easily broken by centrifugation than the adult red cell. It is known that the microcytes in congenital hemolytic anemia are more fragile than normal red cells. In this human disease there is a varying percentage of microcytes and macrocytes in the peripheral blood at different times. The microcytes apparently appear in greater numbers during the periods of crisis when the hematopoietic tissues no doubt are put under the greatest strain. Apparently the hematopoietic tissues of the duck when placed under a severe strain also respond with the escape of small erythroblasts into the blood stream. As far as we know there are no studies on the oxygen-carrying capacity of the microcytes in diseases in man such as congenital hemolytic anemia. It has been shown, however, that duck blood with a high percentage of young erythrocytes is a poor carrier of oxygen.<sup>10</sup> If a corresponding phenomenon does occur in man then we may have an explanation for the disproportion between the clinical manifestations and the degree of anemia frequently observed in this disease.

The rapid increase in the number of erythroblasts preceding the peak of parasitemia in *P. lophurae* infection in ducks is very different from the observations of Terzian on *P. lophurae* infection in chicks. Terzian says that "in view of the rapid

cell destruction taking place during the course of an infection, one would expect an immediate reticulocyte response. It is of interest to note, however, that such a response is singularly lacking and does not occur until some time after the numbers of parasites have begun to decline. . . . Once the animal has succeeded in clearing the circulation of the parasites it is able to regenerate its blood supply very rapidly."<sup>2</sup> Figure 3 shows that the number of erythroblasts in the peripheral blood of ducks is increased with the diminution in the number of erythrocytes within the circulation; furthermore, young cells appear in the peripheral blood before the peak of parasitemia is reached.

Previous experimental studies have shown that the oxygen-carrying capacity of duck blood is decreased when there are many erythroblasts in the circulation.<sup>8,10</sup> The present study also shows that the amount of hemoglobin in duck blood with a high percentage of erythroblasts is less than that in normal blood. Furthermore, the ratio of hemoglobin to cell volume is not proportional to the number of red cells, following the time of the peak of the infection. This, of course, indicates a disproportion between the size of the red cell and a normal amount of hemoglobin for the cell. The illustrations in figure 4 show some large erythroblasts in the peripheral blood following the time of the peak of parasitemia. The percentage of such large cells, however, was not determined in this study.

Histological studies on the spleen and liver of ducks infected with *P. lophurae* have shown a proliferation of cells that were considered to be hematopoietic tissue.<sup>18</sup> The present study of the cells in the liver and spleen would indicate that these hyperplastic foci are formed primarily by cells of the erythrocytic series. The changes that occur in the type of red cell in the peripheral blood, of course, are indicative of the processes occurring within the blood-forming tissue. In some of the birds dying from the infection only very young forms of the erythroblasts are present in significant numbers within the hematopoietic tissues. This, of course, would suggest that the need for red cells in the peripheral circulation in these birds is greater than the ability of the host to produce them. It would seem that ducks with *P. lophurae* infection might not succumb if the hematopoietic tissue always could supply an adequate number of mature erythrocytes. At the time of death young erythroblasts may be present in the peripheral blood. The ducks that have a high parasitemia at the time of death show a large number of erythroblasts with one or more parasites within their cytoplasm. Apparently these plasmodia do not prefer young erythrocytes to adult cells; however, when the adult cells are absent they will enter the younger forms. It may be significant that *P. lophurae* does not prefer the young erythrocytes since the rapid decrease in the degree of parasitemia occurs at the time when the peripheral blood has the maximum number of these young cells. It is suggested therefore that one significant factor in the mechanism by which a rapid decrease in the parasitemia occurs in highly parasitized birds following the peak of infection is the result of an absence of mature erythrocytes for the parasites to enter.

The increase in the color index of the blood from normal ducks given copper and iron would suggest that the anemia in the duck may be influenced favorably by the

administration of large quantities of these elements. However, since it requires approximately two weeks for the blood of normal ducks to show any significant increase in the color index it would be almost useless to give copper and iron to ducks after they are infected with *P. lophurae* in an attempt to influence the anemia during the acute phase of the infection.

The spleen, liver, and bone marrow supply young erythrocytes for the peripheral blood. The data given in table 1 show that the greatest number of the youngest forms of erythrocytes are present in the bone marrow, and furthermore, the young cells in the spleen and liver usually are more mature than those in the bone marrow. In this connection it would be of interest to know if myeloblasts, myelocytes, and polymorphonuclear leukocytes develop similarly in the spleen, liver, and bone marrow in myeloid leukemia in man.

#### SUMMARY

This study of the anemia produced by *P. lophurae* in ducks emphasizes the significance of a decrease in the number of red cells in this disease, and furthermore, it suggests that the rapid diminution in the number of parasites following the peak of the parasitemia may be directly related to the character of this anemia since these parasites apparently do not prefer young erythroblasts to mature erythrocytes.

The observations made in this study show that erythrocytes in the ducks first appear as spherical bodies in stained smears and when mature they are elliptical in shape. Under abnormal conditions such as may occur with a severe parasitemia, erythroblasts appear in the peripheral blood in large numbers, sometimes almost completely replacing the adult type of red cell. Both microcytes and macrocytes appear in the blood stream. The amount of hemoglobin within some of these young erythrocytes is small as indicated by the hemoglobin determinations and the staining reaction. Small young erythroblasts with little hemoglobin appear in the peripheral blood when the maximum load is placed upon the hematopoietic tissues.

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# DIETARY FACTORS CONCERNED IN ERYTHROPOIESIS—Continued

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## IV. MINERALS—continued

In table 9 the average values of the iron content of various tissues under normal conditions, under conditions of prolonged iron deficiency, and following hemoglobin injections are given. These values are taken from the papers of the Rochester group.<sup>384, 385</sup>

TABLE 9.—Distribution of Tissue Iron

Tissue	Parenchyma (Iron Deficiency)	Normal (Standard Diet)	Following Hb <sub>2</sub> Injections
Liver.....	2	25	31
Spleen.....	6	46	105
Rib Marrow.....	12	15	30
Kidney.....	2	5	21
Pancreas.....	2	2	3
Heart.....	2	4	4
Muscle.....	2	4	4
Lungs.....	3	7	6

Values are expressed in mg. per cent.

Little is known concerning the chemical nature of the stored iron. Whether the liver builds the simple precursors into compounds which are transferred to the bone marrow where they are assembled into hemoglobin or whether iron is sent to the bone marrow as a simple salt is not known. Recently several iron-containing compounds have been isolated from liver and spleen as indicated below. These may prove of great interest. Further work on these compounds may well elucidate the mechanism by which iron is incorporated into the hemoglobin molecule.

Approximately 60 per cent of the iron in thoroughly perfused liver is in protein combination, in so far as it is removable from solution by precipitation with trichloroacetic acid. McFarlane<sup>394</sup> has shown that 43 to 60 per cent of the iron in perfused adult rat liver reacts directly with bipyridine in the presence of a reducing agent and must therefore be in some form of chemical combination other than that of the iron in hematin. About 60 per cent of this nonhematin fraction appears in

the trichloroacetic acid precipitate. The total iron in a trichloroacetic acid filtrate reacts directly with potassium thiocyanate in acid solution after oxidation with hydrogen peroxide and this fraction accounts for about 40 per cent of the non-hematin iron. About 50 per cent of the total iron in the trichloroacetic acid filtrate is precipitated along with organic substances by normal lead acetate. The digestion of fetal calves liver, adult rat liver and muscle tissue, and beef spleen by pepsin at about pH 2 produces a five-fold increase in the iron content of the trichloroacetic acid filtrate. The autoproteolytic changes in the liver and spleen at pH 4.5 also include the decomposition of organic iron-containing compounds, presumably iron proteinates. This decomposition is accelerated by hydrogen sulfide and inhibited by copper.

With the knowledge available at the present time one may classify liver and spleen nonhematin iron compounds into the following: (a) ferritin, (b) non-crystallizable ferritins, (c) ferrin, (d) hemosiderin. The portion of the total non-hematin iron present in each of these four compounds is not known.

In 1937 Laufberger<sup>395</sup> isolated the crystalline protein, ferritin, from horse spleen and demonstrated that it contained over 20 per cent by dry weight of iron. The protein was easily crystallized out as the cadmium salt and was found to be stable between pH 4 and 10. Kuhn, Sörenson, and Birkofer<sup>396</sup> concluded that ferritin consists of 54.5 per cent protein, 12.1 per cent nucleic acid, and 35 per cent  $\text{Fe}^{+++}\text{OOH}$ . Careful and thorough studies on the chemical, physical, crystalline, and magnetic properties of ferritin have been performed by Granick, Rothen, Michaelis, and Coryell and their results are reported in a series of papers.<sup>391, 397-403, 664</sup> Horse spleen ferritin is a protein easily crystallizable as the cadmium salt and contains over 20 per cent iron. Ferritin can be freed from its iron by reduction to the ferrous state and removed by dialysis after combination with  $\alpha$ -bipyridine. An iron-free, colorless protein solution results, from which the protein crystallizes in the presence of cadmium sulfate in the same crystal form as does ferritin. This colorless protein is designated as apoferritin and has been investigated in the ultracentrifuge and shown to be a very homogeneous protein with a molecular weight of 465,000. It was found by solubility studies, variation in the iron and phosphorus content, and by studies in the ultracentrifuge that ferritin is not a definite molecular species but consists of a mixture of a complex of apoferritin-iron hydroxide and about 25 per cent free apoferritin, the mass of these particles not being uniform. It was concluded that the iron of ferritin was most likely present in the form of micelles of ferric hydroxide interspersed in the apoferritin crystal lattice in the spaces between the protein molecules. The approximate composition of the iron-rich micelles of ferritin appears to be  $(\text{FeOOH})_8 (\text{FeO}-\text{OPO}_3\text{H}_2)$ . Magnetic measurements have shown that the iron in the micelles is present in the rarely occurring state of 3 unpaired electrons per iron atom. These workers were unable to confirm the presence of nucleic acid in ferritin.

Ferritin is widely distributed among mammals and the species from which it has been isolated are, in order of decreasing ferritin content, horse, man, dog, guinea pig, mouse, rat, pig, rabbit, and cat. The organs from which it has been isolated are spleen, liver, bone marrow, kidney, and testicle. No trace of ferritin

could be demonstrated in red or white blood cells or blood plasma. In man, ferritin has been found in the spleen, liver, and bone marrow. The total amount and concentration of ferritin in human liver is much greater than that in the spleen. It may be significant that extensive bleeding in horses lowers the ferritin and apoferritin content of the spleen. The decrease in the apoferritin content suggests a reutilization for blood production of the protein fraction of the ferritin as well as of the iron. That ferritin is found in high concentration in the red bone marrow is also suggestive of a role in blood formation. The function of ferritin as storage iron has been shown in dogs using the radioactive isotope. Radioactive iron in the form of ferric ammonium citrate when administered by vein is readily converted into ferritin iron in the liver. One hour after injection over 40 per cent of the injected iron was found to be present in the ferritin-rich fraction. In another dog, after 2 hours, 61 per cent of the injected iron was found in this fraction. It has also been demonstrated that iron liberated from destroyed red cells is used for the construction of new ferric hydroxide micelles of ferritin. The authors have therefore concluded that "ferritin iron acts in the capacity of storage iron in the animal body."

The *in vitro* conversion of radioactive iron to ferritin has been studied and it has been found that ferritin may take up almost 50 per cent of radioactive iron by mixture with ferric ammonium citrate. The incubation of guinea pig liver brei with apoferritin and radioactive ferric ammonium citrate, the subsequent isolation of crystalline ferritin, and the determination of its radioactivity indicate that iron micelles of ferritin are the result of metabolic synthesis, perhaps due to a specific enzyme.

After ferritin is removed by crystallization with cadmium sulfate, there remains a brown mother liquor which cannot be made to yield further crystals but contains a brown, colloidal, nondialyzable compound containing about 19.8 per cent iron and somewhat less nitrogen than ferritin although the relative nitrogen, phosphorus, and iron values do not differ appreciably from ferritin. It is thought that the iron is present as a colloidal ferric hydroxide, the micelles of which contain in part ionic constituents other than OH, and that these micelles are loosely attached to proteinaceous material, including some denatured apoferritin. This fraction is called "noncrystallizable ferritin" and its significance is unknown.

Libet and Elliott<sup>404</sup> have described an iron-protein complex in liver which they have termed ferrin. This compound differs from ferritin in several respects. It precipitates rather than crystallizes on addition of cadmium sulfate, it precipitates at a lower concentration of ammonium sulfate than does ferritin, it is not present in appreciable quantities in the spleen, and the iron in ferrin is more active than the iron in ferritin in the catalysis of phospholipid oxidations.<sup>405</sup> It is not known whether ferrin is a single and specific compound, nor is it known whether or not it represents a denatured product. The function of ferrin has not been studied.

The yellow brown granules seen in the tissues of most mammals have long been known as hemosiderin. The most unusual feature of this iron-containing compound is the appalling lack of knowledge concerning it. It is recognized that it is the iron-containing portion of hemoglobin which rests in the cells of most tissues but es-

pecially is it found in the spleen, liver, and kidney following the destruction of erythrocytes. Hemosiderin probably represents some stage or side reaction in the disintegration of hemoglobin into bile pigment but the exact nature of the reactions involved is not understood. There is little doubt that it functions as storage iron, for the granules are abundant following repeated injections of ferric chloride<sup>406, 407</sup> and disappear when the demands of the body for iron are great. The precise chemical nature is unknown. Cook<sup>408</sup> in 1929 presented evidence that the iron-pigment consists of organic granules impregnated with ferric oxide. The iron can be removed by treatment with acid, leaving the substrate practically intact. This iron then reacts with thiocyanate and other substances in a manner which is not characteristic of ionic iron. Recent work by Michaelis and co-workers<sup>399</sup> casts doubt on the homogeneity of hemosiderin granules. They conclude that the iron of hemosiderin is, "at least in the main part, in the same magnetic state as ferritin, and that some of its iron may be in a state of lower susceptibility." Hemosiderin granules isolated by differential centrifugation in a partial state of purity have an iron content of 8.29 per cent, a nitrogen content of 12.9 per cent, and a phosphorus content of 1.6 per cent.<sup>408</sup>

Saha and Guha<sup>409-412</sup> have obtained an iron-copper-nuclei-protein complex in a fairly pure state from fish. They suggest on a basis of the hemoglobin-regenerating potency of this compound in anemic rats that it may be a precursor of hemoglobin.

5. *Anemia of iron deficiency.*—The anemia of iron deficiency and its response to iron therapy are well recognized and have been described many times.<sup>309, 413, 414</sup> Only certain features will be summarized here.

A hypochromic, microcytic anemia is characteristic of iron deficiency and has been described in many different species. In the advanced stages marked anisocytosis and poikilocytosis of the red cells are present. The mean diameter of the red cells is small and the mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin are reduced. The Price-Jones curves in severe cases show a marked shift to the left with a broad base. Reticulocytes, punctate basophilia, and nucleated red blood cells are occasionally seen. The white blood cells and platelets are usually unaffected.

In the absence of iron, hemoglobin cannot be synthesized and there is a maturation arrest at the normoblastic stage. The bone marrow is hyperplastic and shows a predominance of the more mature nucleated red blood cells, principally the polychromatic normoblasts.<sup>320, 415-417</sup>

There is no evidence that there is an increased rate of destruction of red cells. The serum bilirubin and icterus index are either normal or diminished and hemoglobin destruction as measured by urobilinogen excretion is diminished.<sup>418</sup>

Porphyryn metabolism has been inadequately studied in iron deficiency. Duesberg<sup>419</sup> states that porphyrin excretion is diminished and on this basis suggests that iron may function in the preporphyrin stage of hemoglobin formation. Seggel<sup>420</sup> has shown that erythrocyte protoporphyrin is increased in iron deficiency and this has been clearly confirmed by Watson et al.<sup>421</sup> The normal erythrocyte protoporphyrin ranges between 20 and 45  $\gamma$  per 100 cc. of erythrocytes. In a woman with a marked hypochromic anemia due to chronic blood loss from bleeding

hemorrhoids (case no. 12, Watson et al.) protoporphyrin was found to be as high as 613  $\gamma$ . Following iron medication this fell to 40  $\gamma$ . These authors assume that the increase is a result of cessation of hemoglobin formation at the porphyrin stage.

Moore, Doan, and Arrowsmith<sup>350</sup> and others<sup>422</sup> have demonstrated that the serum iron level is low in iron deficiency anemias of varied etiology. There is no consistent alteration of the "easily split-off" iron under these conditions. Under the influence of iron therapy, serum iron values return to normal as the mean corpuscular hemoglobin concentration increases to normal and as the state of iron deficiency is corrected.

6. *Excretion*.—It has long been claimed that iron is excreted by three routes—urine, bile, and the large intestine. The older methods of iron analysis were fraught with many difficulties. Since the use of radioactive iron much more reliable information is at hand and the older concepts will have to be altered.

It is now generally agreed that only a minimal amount of iron is excreted in the urine. It is questionable whether this small amount has any significance. It is probably derived from cellular debris. It has been shown that the urinary excretion of iron is rather constant in the same individual and that it is not related to iron intake. Barer and Fowler<sup>423</sup> have summarized the literature and report the average value for 100 men to be 0.395 mg./day and for 100 women 0.489 mg./day. In rats Greenberg, Copp, and Cuthbertson<sup>424</sup> were able to demonstrate that 1.5 per cent of the administered dose of radio-iron was excreted in the urine. Habs and co-workers<sup>425</sup> injected radio-iron intravenously in dogs and found that only small quantities were excreted by this route following iron injections. Later the excretion dropped to traces and even to zero. These workers suggested that the small quantities found immediately after the injection were derived from the diffusible fraction of plasma iron. Similar conclusions have been reached by Little, Power, and Wakefield.<sup>426</sup>

There is evidence that significant amounts of iron are eliminated in the bile under certain circumstances. However, under normal conditions iron is eliminated in the bile of dogs at the low but constant rate of 0.2 mg./day.<sup>427</sup> The excretion is not increased by the injection of iron by vein. Confirmatory results have been obtained in the rat using the same method.<sup>428</sup> Under conditions of increased blood destruction<sup>427</sup> the elimination of biliary iron may increase ten-fold and parallels the increased output of bile pigment, but even then only 3 per cent of the released iron is eliminated in the bile.

Welch, Wakefield, and Adams<sup>428</sup> were the first to challenge the older concept that the intestine holds the power to regulate the excretion of iron. They showed in a patient with an ileostomy that the excretion of iron into the colon was negligible. McCance and Widdowson<sup>339, 340</sup> and Fowler and Barer<sup>429</sup> by introducing iron parenterally came to the same conclusion. McCance and Widdowson<sup>430</sup> further demonstrated that less than 0.5 per cent of the total amount of iron liberated by the destruction of red cells with acetyl-phenylhydrazine in a polycythemic patient was excreted. Recently these authors<sup>431</sup> have studied iron excretion in a patient with a hemolytic anemia who received approximately 80 mg. of iron per day

intravenously in the form of transfusions for 100 consecutive days. In addition to this, her daily dietary intake of iron was 5.6 mg. per day. On this regimen of 85.6 mg. intake of iron per day she excreted only 5.2 mg. total per day in urine and stools. From this the authors concluded that she was unable to excrete the large quantities of superfluous iron and that iron once absorbed remains in the body. Histologic study by Maddox and Heath<sup>432</sup> of the gastrointestinal tract and of a colonic explant on the abdominal wall of dogs before and after the administration of iron revealed no evidence that iron can be observed in the process of excretion by these organs. Work with intravenously administered radioactive iron has confirmed this view. Hahn and co-workers<sup>425</sup> found that in 5 dogs receiving 100 to 250 mg. of radio-iron the fecal excretion settled down to 0.05 to 0.4 mg. per day. Greenberg et al.<sup>424</sup> in a similar experiment in rats found 1.9 per cent of the radio-iron in the gastrointestinal tract. Both groups of workers suggest that this small quantity may be derived from epithelial wastage.

In summary it may be said that small but insignificant amounts of iron (approximately 1 mg. per day) are excreted by the body through the urine, bile, and intestine. It would seem that the body controls the iron stores by controlling the absorption rather than its elimination and that once iron gains entrance into the body it remains there.

**B. Copper.**—There has been no subject in hematology more controversial than that of copper. The earlier literature concerned itself principally with the effectiveness or ineffectiveness of iron salts in the prevention and cure of the anemia produced in rats maintained on a milk diet. This controversy resulted in a voluminous literature. It has now been demonstrated many times in several different species that copper is needed in addition to iron in order either to prevent or to cure the anemia. This earlier literature has been reviewed by Elvehjem.<sup>433</sup>

Unfortunately, so much emphasis has been placed on the value of copper in the treatment of experimental anemia in rats and in certain instances of iron deficiency anemia in human beings that, although the presence of copper in normal tissues has been recognized for a long time, little attention has been given to its function and metabolism. Since the original controversy interest in this element has waned. This is extremely unfortunate since an understanding of copper metabolism is essential to an understanding of the process of erythropoiesis.

Various reviews on the subject of copper are available.<sup>305, 308, 433-436</sup>

1. *Copper deficiency in animals.*—In 1924 a series of studies was begun at the University of Wisconsin which demonstrated that when rabbits or rats were raised on a diet consisting of whole milk an anemia developed.<sup>437, 438</sup> This anemia failed to respond to highly purified iron salts but did respond to the ash of liver, lettuce, or corn especially when supplemented with an iron salt.<sup>439, 441</sup> The pale blue color of the liver ash suggested that copper might be the active factor.<sup>440</sup> It was soon discovered that the addition of 0.05 mg. of copper together with 0.5 mg. of iron to the whole milk diet produced an immediate and striking recovery.<sup>442</sup> Iron alone failed to cause a reticulocytosis while copper alone produced a small prolonged response. When both iron and copper were given a reticulocytosis of 16 per cent developed in 4 days.<sup>443</sup> The minimal daily requirements for the production of a typical reticu-

locyte response in an anemic rat were found to be approximately 0.3 mg. of iron and 0.005 to 0.01 mg. of copper.<sup>443</sup> Manganese as well as eleven other elements were found to be inactive.<sup>444</sup> Underhill, Orten, Mugrage, and Lewis<sup>445</sup> demonstrated that rats maintained for 667 days on a milk diet supplemented with iron and copper maintained a normal blood picture.

Smith and Medlicott<sup>451</sup> have made a detailed morphological study of the red blood cells in rats deficient only in copper and have found that the anemia is microcytic and hypochromic and is accompanied by a moderate reticulocytosis of 8 per cent. Blood smears showed a microcytosis, hypochromia, and occasional basophilic red cells and poikilocytes. The microcytosis was not as marked as in iron deficiency anemia. The feeding of copper to rats deficient in both iron and copper produced a marked reticulocytosis, a rise in the erythrocyte count, and no change in hemoglobin. This work is summarized in table 10.

Copper has been shown to be essential for erythropoiesis in dogs.<sup>449,450,498</sup> This conclusion has been questioned by Whipple and his group.<sup>319,308</sup> They found that

TABLE 10.—*Summary of the Blood Picture of Normal and Anemic Rats*

Treatment	R.B.C. Millions per cu. mm.	Hbg. Gm. %	Ht. cc. per 100 cc.	MCV cu $\mu$	MCH m $\mu$	MCHC %	Retic. %
Normal.....	7.4	14.9	44.7	61	33	20	3
Milk Anemia.....	3.2	3.4	11.5	37	28	11	9
Fe—Fed.....	2.8	3.6	13.5	51	27	14	8
Cu—Fed.....	4.2	3.4	14.6	35	23	8	24

Modified from Smith and Medlicott.<sup>451</sup> Average values are given; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

dogs made anemic by bleeding have abundant stores of copper in the liver and spleen and that the administration of copper has only a moderate or irregular effect on hemoglobin production. They state that they have no explanation for this indefinite and irregular response. Yet, they admit that the salmon bread diet used by them supplies at least 1 mg. of copper daily. Since copper is not a constituent of hemoglobin as is iron and whole blood contains only approximately 150 micrograms per 100 cc., phlebotomy draws only little on the copper stores. Therefore, there is no reason to expect that their dogs should have been copper deficient or that they should have responded to copper therapy. When growing dogs are maintained on a copper-low milk diet and then phlebotomized, it has been adequately demonstrated that they are copper deficient and consequently respond well to iron only after the addition of copper.<sup>449,450,498</sup>

In copper deficiency anemia in dogs it has been reported that there is no significant alteration in the mean corpuscular volume or in the saturation index.<sup>450</sup> However, during copper therapy the mean corpuscular volume increases inconstantly with the hemoglobin increase.

A condition called "enzootic ataxia" and variously known as "Gingin rickets" or "ataxia in young lambs" has been described in sheep in Western Australia.<sup>452</sup>

Diseases of lambs with similar clinical features have been described from other parts of the world as "renguera,"<sup>453</sup> "paralysis of lambs,"<sup>454</sup> "sway-back," "swing-back," and "warfa."<sup>455,456</sup> These conditions have been incompletely studied and their etiology is unknown.

The condition occurring in England and known as "sway-back" responds to copper treatment even though it has been demonstrated that the grasses in the affected areas and the livers of the affected animals are not deficient in copper.<sup>509,510</sup> It has been suggested that the disease is due to a disturbance of copper metabolism.<sup>457</sup>

The condition occurring in Western Australia has been carefully studied and conclusively shown by Bennetts and Beck<sup>452</sup> to be due to copper deficiency. A complete review of their work is worth while since it demonstrates in detail the manifestations of copper deficiency in sheep and shows so clearly the relationship of copper to hematopoiesis. Manifestations have been shown to be present in both pregnant or lambing ewes and in lambs. The disease occurs most commonly in lambs of 1 to 2

TABLE 11.—Blood Values for "Normal" and Affected Lambs\*

Subjects	R.B.C. X10 <sup>6</sup> per cu. mm.	Hbg. Gm. %	Ht. cc. per 100 cc.	MCV cu. $\mu$	MCH $\mu$ m	MCHC %
12 Healthy Lambs from "Sound" Localities	(14.0-16.9) 15.0	(13.2-17.6) 15.7	(41-47) 44	(27-32) 30.0	(9 -12.0) 10	(29-43) 35
3 Ataxic Lambs	( 9.4-17.1) 12.1	( 5.9-13.2) 8.5	(19-37) 25	(20-22) 21	(6.0- 8) 7	(30-35) 33

\* Modified from Bennetts and Beck.<sup>22</sup> MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration. Note: The MCH and MCHC were calculated from the data by the reviewer. Figures in parentheses represent range. Lower figures represent mean.

months of age. The first manifestations are "an appearance of unthriftiness" and retardation of growth. Incoordination of the gait affecting the hind limbs soon follows. The animals develop marked ataxia and eventually the forelegs are affected. They then remain in a position of decubitis and die in this condition in 3 to 4 weeks from malnutrition and intercurrent infections. The condition appears to be essentially one of ataxia without true paralysis. Pathologically the disease is characterized by demyelination of the nervous system, a typical degeneration of the myelin sheaths of nerve fibers in the cord being pathognomonic. Both motor and sensory fibers are involved. Extensive brain lesions especially in the medulla and cerebellum may occur. Hemosiderin was commonly seen in the livers and occasionally in the spleen and kidneys of the lambs. A definite hypochromic, microcytic anemia was frequently seen in the subacute disease. This is demonstrated in table 11. Blood smears showed anisocytosis and hypochromia. Stippled cells were rarely observed and were never numerous. The administration of copper to these animals completely alleviated the anemia. There was a striking correlation between the degree of anemia in the late stages of pregnancy or the early lactation period in the

ewe and the development of ataxia in the lamb. A correlation was demonstrated between low blood copper in the mother and the occurrence of ataxia in the progeny. No definite relationship was found between the level of hemoglobin and blood copper in the lambs although low hemoglobin values were not noted until the blood copper had fallen to levels between 0.01 and 0.02 mg. per cent. In many cases the blood copper fell to these levels without a fall in the hemoglobin value. When the hemoglobin diminished it was usually some time after the copper level had fallen. Chemical analysis of the liver, blood, and milk of the mothers and of the liver and blood of the affected progeny demonstrated conclusively and constantly in all cases analyzed that a low copper status existed. Analyses of pastures in the affected districts were extremely low in copper as compared with pastures in nonaffected districts. The administration of copper to the mother during the gestation period prevented completely the occurrence of the disease. Copper administered to the lambs arrested the disease, even in the later stages, at which time the course was rapid in untreated controls. Treated animals, apart from persistence of some ataxia, recovered. Copper supplements in the form of a lick or as a top dressing prevented the occurrence of ataxia and promoted optimal growth in lambs.

The manifestations seen in adult sheep were somewhat different from those observed in lambs. "Stringiness" of the wool was observed in the animals after grazing a few months on copper-deficient pastures. Ataxia and pathological changes in the nervous system were not observed in adult sheep. There was, however, moderate hemosiderosis of the liver, kidneys, and spleen. The degree of hemosiderosis appeared to be related to the degree of anemia. The breeding ewe exhibited diarrhea and anemia. The anemia was severe. Values of as low as 2.7 Gm. per cent hemoglobin, 2.7 million red blood cells, and a volume of packed red blood cells of 15.5 cc. per 100 cc. were observed. The blood studies are summarized in table 12. In severe cases macrocytes were sufficiently numerous to raise the mean corpuscular volume significantly above normal. In spite of this slight macrocytosis a mild hypochromia existed. The authors state: "A classification of the anemia is not possible without further extensive investigations which are beyond the scope of the present inquiry." Examination of blood smears revealed marked anisocytosis, poikilocytosis, numerous macrocytes, stippled cells, and polychromatophilia. Howell-Jolly bodies and normoblasts were occasionally seen. In 1 case 49 normoblasts per 100 white blood cells were observed. In 2 cases "macroblasts" were seen. Reticulocytes were generally numerous in the more anemic animals (mean 4.2 per cent and maximum 11 per cent). As in the case of the lambs the anemia was associated with low copper values in the blood and liver. Very low blood and liver copper values were sometimes present in the absence of anemia and anemia generally developed several months after the blood copper had fallen to very low levels. After the completion of gestation and lactation periods the blood copper levels returned to normal earlier than the hemoglobin values, but low liver copper levels persisted. The anemia in all cases responded to "pure" copper salt supplements with a reticulocyte peak of approximately 20 per cent occurring on the 5th to the 7th day. Thereafter the blood picture steadily improved, reticulocytes and other abnormal cells disappeared from the circulation and by the fourth week the red

blood cell count, hemoglobin, and mean corpuscular volume approached normal values. During the same period control ewes became somewhat more anemic. The authors conclude, "It is clearly evident that in sheep as in other species copper is necessary for normal hemoglobin formation and erythropoiesis. It would appear, however, that this function may be carried out, in the sheep, under conditions of copper deficiency when the blood and liver copper values of the animal are very low, provided that it is not called upon to produce and rear progeny. In this event, owing to the drain on the mother's reserves for the embryo a breakdown may occur and anemia supervenes."

An enzootic disease of cattle is known to occur in the areas in which the enzootic disease in sheep is prevalent. This condition in cattle, known as "falling disease" because of its termination in sudden death, has been studied in detail and reported

TABLE 12.—Blood Values of "Normal" and Affected Sheep\*

Group	R.B.C. $\times 10^4$ per cu.mm.	Hbg. Gm. %	Ht. cc. per 100 cc.	MCV cu. $\mu$ .	MCH $\mu$	MCHC %
"Normal"	(7.7-13.2) 10.4	(9.8-16.8) 12.8	(30.0-48.8) 36.5	(30-39) 35	(11-14) 12	(33-43) 35
"Nonanemic"	(6.7-13.1) 9.9	(8.3-15.8) 11.7	(20.0-42.5) 35.2	(29-42) 36	(10-14) 12	(28-47) 33
"Anemic"	(2.7- 7.4) 5.05	(3.9- 7.5) 5.9	(15.5-24.9) 20.9	(30-59) 43	(11-15) 12	(22-32) 27

\* Summarized from Bennetts and Beck.<sup>452</sup> MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration. Note: The values for MCH and MCHC were calculated by the reviewer from the data. The "normal" sheep consisted of animals taken from healthy areas. The "nonanemic" sheep were animals taken from disease-producing areas and received copper supplementation. The "anemic" animals were breeding ewes from a disease-producing area and had received no copper. Figures in parentheses represent range. Lower figures represent mean.

by Bennetts and his co-workers.<sup>458, 459</sup> Clinically the disease is characterized by a "rough staving coat," a depraved appetite, suppression of oestrus, anemia, and sudden death. Young animals show marked evidences of malnutrition and abnormal development; intermittent diarrhea and anemia are frequently present. Pathologically the disease is characterized by atrophy and scarring of the myocardium, marked hemosiderosis of the liver, spleen, and kidneys, and by an unusual type of glomerulonephritis in the fatal cases.<sup>460</sup> The sudden death would appear to be a result of myocardial failure. The anemia has a seasonal incidence, being especially prevalent in September and October, it may be exceedingly severe, and it is reported to be definitely macrocytic and somewhat hypochromic. The degree of anemia correlates well with the degree of reduction of the blood copper. Anisocytosis, poikilocytosis, numerous macrocytes, polychromasia, punctate basophilia, and occasional Jolly bodies were seen in the blood smears. Reticulocytes were not numerous. Evidences of increased cell fragility or excessive hemolysis were not found. A low

copper status in the liver and milk of the animals as well as a low copper content of the pastures has been demonstrated. Copper supplements prevented the appearance of the seasonal anemia as well as the other clinical signs of the deficiency and alleviated the anemia, promoting optimal health in affected animals maintained in affected areas. In an experimental dairy herd optimal response was obtained by the administration of pure copper supplements; the addition of other minerals had no appreciably beneficial effect. Thus it would appear that "falling disease" in cattle is due to an uncomplicated copper deficiency.

In addition to rats, mice, rabbits, dogs, sheep, and cattle copper has been shown to be essential for erythropoiesis in chickens<sup>446</sup> and pigs.<sup>447,448</sup> Elvehjem has concluded "that copper is of fundamental importance in the formation of hemoglobin in all red-blooded animals."

2. *Copper deficiency in man.*—In the treatment of nutritional anemia in infants it has often been demonstrated that when iron therapy is combined with copper therapy the rate of hemoglobin formation is more rapid than when the anemia is treated with iron alone. Josephs<sup>461</sup> treated a large series of infants with iron and copper. The hemoglobin curves of the group treated with iron tended to flatten out at about 50 per cent, whereas the curves representing the infants on both iron and copper continued to rise steeply to about 70 per cent. Hutchinson<sup>462</sup> treated 9 anemic infants for periods of 1 to 5 weeks with ferrous sulfate. Medication was then discontinued for 1 to 6 weeks. At the end of this time the hemoglobin values had become stationary. Copper sulfate was then given. In every case there was a further rise in hemoglobin. Similar results have been reported in Parsons and Hawksley<sup>463</sup> in 3 cases, by Elvehjem, Duckles, and Mendenhall<sup>464</sup> in 6 cases, and by Lewis<sup>465</sup> in 29 cases, as well as by others.<sup>466-469</sup>

Usher, MacDermot, and Lozinski<sup>470</sup> tested the efficacy of copper as a prophylactic agent against the anemia of infancy in 233 institutionalized patients. They found that at the age of 1 year, the group receiving both copper and iron supplements had an average hemoglobin value of 19 per cent above that of the control group receiving no supplement, whereas the group receiving iron alone had an average hemoglobin value 15 per cent higher than that of the controls.

An analysis of the livers of anemic infants has revealed a lower copper content than the livers of nonanemic controls.<sup>471</sup> A reduced copper content of the livers of infants during the nursing period and an increase as soon as a mixed diet was supplied has been reported.<sup>472</sup> The most convincing proof of the efficacy of copper is presented by Moore.<sup>435</sup> Using the double reticulocyte method, he treated hypochromic anemia in infants with ferrous sulfate. After 2 weeks, copper sulfate was added to the iron. In several cases the copper initiated a second reticulocyte rise.

There is very little evidence to indicate that iron alone is as effective as combined iron and copper therapy in infants. That evidence which does exist is not convincing. Mackay<sup>473</sup> found that iron and copper were no more effective in 6 cases with nutritional anemia than in 6 cases given iron alone. The iron preparation used in her series, as she admits, was not copper free. Lottrup<sup>474</sup> obtained results similar to Mackay's in 2 cases.

Elvehjem, Duckles, and Mendenhall<sup>464</sup> make the statement: "Since milk is low

in both iron and copper, 1 liter of certified milk containing from 0.12 to 0.18 mg. of copper and approximately 0.50 mg. of iron, it is not surprising that the reserves of infants may become depleted and that anemia may result."

The value of copper in the treatment of hypochromic microcytic anemia in adults is controversial. Mills<sup>475</sup> reported cases which did not respond satisfactorily to 15 to 60 grains of "Blaud's mass" daily for a period of one to several months but did respond rapidly when copper was added to 75 grains of Blaud's mass. Five cases which were treated initially with Blaud's mass containing copper responded both immediately and favorably. Adamson and Smith reported similar results in 10 cases.<sup>476</sup> Dameshek<sup>477</sup> found that about 75 per cent of the cases which he treated responded maximally to a copper-free iron preparation alone; in the remainder small doses of copper sulfate, given with iron, were effective in continuing the hemoglobin response. Waugh<sup>478</sup> in referring to hypochromic microcytic anemia in adults makes the statement: "I am inclined to take the view that there is a more or less specific therapeutic value in the combination of copper with iron in the treatment for this condition," but he presents evidence in only 1 case. Gross<sup>479</sup> treated 8 patients intravenously with a combination of iron cacodylate and copper formate and found this combination effective but observed no controls with the iron cacodylate alone. Jones<sup>480</sup> states that he has encountered many patients who do not respond to either iron or iron and acid until copper is added, but he presents no data.

It is the general consensus of most hematologists that adult patients with hypochromic microcytic anemia, in the absence of infections, malignancy, and continued hemorrhage, will respond satisfactorily and rapidly to iron medication.<sup>485, 481-483</sup> Bethell et al.<sup>484</sup> treated a series of patients with minimal amounts of purified iron in conjunction with a low copper diet and obtained a good rate of hemoglobin formation. The low copper intake apparently made no difference in the response. Fowler and Barer<sup>485</sup> divided the treatment of 19 patients with hypochromic microcytic anemia into three periods. During the first iron was administered, during the second iron plus copper, and during the third the copper was omitted and the dose of iron increased. The average increase in hemoglobin in grams per cent during the first period was  $+0.415$ , during the second  $+0.233$ , and during the third  $+0.336$ . These authors concluded that copper sulfate did not increase the effectiveness of the iron.

From the data presented it is possible to draw certain general conclusions regarding the efficacy of copper in the treatment of microcytic hypochromic anemia in man. In evaluating the literature several factors must be considered. First, it is very probable that a few of the cases in which negative results were obtained were not actually cases of nutritional anemia. Secondly, there may have been factors present such as infections or malignancy which retarded or prevented a response. Thirdly, some of the negative results might be explained on the basis of contamination of the iron with copper or the hospital diets may have contained sufficient copper to make any beneficial effects from added copper difficult to detect. Admittedly, such experiments are difficult to control since the quantity of copper needed is so small. Fourthly, some of the cases presented as not responding to iron alone were probably not given a sufficiently long period of trial and when copper was added the response

may have been due to the iron alone. Reticulocyte counts were not done in any of the cases recorded as not responding to iron.

In nutritional anemia in infants the rate of hemoglobin formation is accelerated when copper is given in addition to iron. In adults supplemental copper therapy may be of value in a few cases. These, if they occur, are rare. Most cases will respond if adequate doses of iron are given. This does not necessarily indicate that copper is not needed for hemoglobin formation or that it is not a dietary essential but rather that the quantities needed are so small that sufficient copper is present in the body stores in adult life, in the diet, or as a contaminant in the iron to supply the needs. No uncomplicated or clearly defined case of copper deficiency in man has been reported.

There is a need for careful studies on the treatment of hypochromic microcytic anemia in adults in which the diet as well as iron used is free from copper and in which use is made of the double reticulocyte method to determine the degree of response to iron as well as the response to copper. Considering the amount of debate which this controversy has caused it is amazing that such studies have not been done. The opinions which have been expressed are based more upon general impressions and poorly controlled experiments in a small number of cases than upon reliable experimental data.

3. *The metabolism of copper in animals.*—Copper is abundant in food<sup>433, 436</sup> and its availability is not a problem.<sup>437</sup> It has been shown that the copper in many naturally occurring compounds is readily utilized by the body. Very little is known concerning the mechanism or site of absorption. The absorption of copper from the upper third of the jejunum has been demonstrated.<sup>492</sup> When the copper stores of rats are markedly depleted only about 5 per cent of the dietary copper is retained.<sup>488-490</sup> This is more, however, than is retained by iron-deficient rats and is a sufficient amount to give rise to maximal hemoglobin formation.<sup>489</sup>

Following the ingestion of radioactive copper by the dog, the element appears quickly in the plasma and continues to rise for 2 to 5 hours, after which it falls abruptly.<sup>491, 492</sup> Sachs et al. have obtained similar results following the oral administration of copper sulfate.<sup>493</sup> Thus plasma is concerned with the transportation of copper. The plasma copper is bound loosely to protein, principally the albumin fraction. About two-thirds of it is split off by cold trichloroacetic acid and very little of it is dialyzable, suggesting that it is in a unionizable form.<sup>355, 491</sup> Within 24 hours the radioactive copper can be demonstrated in the bone marrow as well as in the circulating red cells.<sup>489, 491, 492</sup> The amount in the red cells continues to increase for 24 hours. The total activity in the blood represents about 45 to 65 per cent of the total activity in the body. The relative retention of radio-copper is greatest in the kidney and the liver but is present in almost all tissues. The feeding of iron and copper for a short period of time to severely anemic animals deficient in both iron and copper does not lead to an accumulation of copper in the bone marrow<sup>488</sup> but its presence there has been demonstrated.<sup>489</sup> There is suggestive evidence that in animals in which hematopoietic activity is accelerated the uptake of copper by the red cell is somewhat increased.<sup>491</sup>

The copper content of the whole blood of pigs as well as most other animals is

in the range of 100 to 180 micrograms per 100 cc.<sup>488, 494-497</sup> Copper is distributed approximately equally between the cells and plasma. In nutritional anemia in pigs<sup>497</sup> the copper content of the whole blood falls to levels as low as 7 micrograms per cent and that of the plasma to 4 micrograms per cent. The feeding of small amounts of copper results in a very rapid rise. Schultze, Elvehjem, and Hart<sup>497</sup> have suggested that in the pig rapid hematopoiesis cannot take place unless the copper content of the blood is maintained above 20 micrograms per 100 cc. of whole blood. Dogs made anemic by excluding iron and copper from their diet show a temporary increase in whole blood copper when iron alone is fed.<sup>498</sup> The administration of copper alone is associated with a rise to normal of the blood copper level but the latter falls again in time if the administration is not continued. If then iron is given, there is a sharp rise in blood copper. Thus, when an acceleration of hematopoiesis takes place there is a mobilization of copper from the stores to the blood, provided such stores are available. This is in accord with the observations that following hemorrhage there is an increase in blood copper.<sup>499, 500</sup>

The storage of copper in the tissues, especially the liver, has been studied in detail by many investigators. The results of this work may be briefly summarized. During embryonal development there is a high concentration of copper in the liver.<sup>501, 502</sup> This increases until birth, at which time it is higher than any other time in the life of the animal.<sup>503, 504</sup> Since the copper content of milk is low the reserves of this element diminish during the nursing period.<sup>505</sup> If the animals are then continued on a milk diet the copper content of the tissues is severely depleted.<sup>488, 490</sup> The feeding of copper results in the restoration of these reserves.<sup>490, 504, 506</sup> It seems that in the stages when the liver is most actively engaged in hematopoiesis it contains the greatest amount of copper. The feeding of a high copper diet to mothers has very little effect on the fetal stores.<sup>504, 507</sup> Restriction of copper in the mother results in depletion of the fetal reserves.

There is very little information available regarding the excretion of copper. Using radioactive copper Schultz and Simmons<sup>549</sup> found appreciable amounts in the urine. How much of this was due to contamination with the feces they were unable to state. Lindow, Peterson, and Steenbock<sup>505</sup> detected copper in the urine of rats maintained on stock rations. When this ration was supplemented with copper the urinary excretion increased five-fold. However, 98 per cent of the added copper was excreted in the feces. Schubert and co-workers<sup>192</sup> using the radioactive isotope report that the liver is the chief excretory organ.

Eden<sup>511</sup> has studied the excretion of copper in the rabbit. Following oral administration he found that about 0.2 per cent was excreted in the urine. When the copper was placed directly into the stomach approximately 1 per cent appeared in the urine. After an intravenous injection the copper content of both the urine and the feces rose sharply. Sandberg and Perla<sup>512</sup> splenectomized rats and noted an increased elimination of copper in the feces, which commenced two weeks after splenectomy. This was associated with a persistent negative nitrogen balance and they concluded that the spleen is essential for the utilization of copper.

1. *Metabolism of copper in man.*—The absorption and excretion of copper in man has been little studied. Tompsett<sup>513</sup> noted a daily excretion of 0.63 mg. of copper in

a patient receiving 0.21 mg. daily. Chou and Adolph<sup>471</sup> studied the copper metabolism of 4 normal adults for periods of 1 to 3 days and found that equilibrium was reached on a daily intake of about 2.0 mg. of copper. Ohlsen and Daum<sup>514</sup> found that when the intake of copper for 3 young women ranged from 0.96 mg. to 1.13 mg. the excretion exceeded the intake by amounts of from 0.08 to 0.40 mg. Leverette and Binkley<sup>515</sup> found that the average daily intake of copper of 65 normal young women on self-chosen diets was 2.65 mg. The average daily retention by this group was 0.85 mg. As the copper intake increased the percentage retained increased. In general about 20 to 35 per cent of ingested copper is retained. It may be concluded from these balance studies that the daily requirement of copper for adults is 2.0 to 2.5 mg. This amount is easily obtained from normal diets.

In 3 normal preschool age boys Scoular<sup>516</sup> has reported that only 15 to 58 per cent of the ingested copper is excreted by way of the alimentary tract and that the daily urinary copper excretion was fairly constant, averaging 4 per cent of the ingested copper. Daniels and Wright<sup>517</sup> in similar subjects found a total excretion of 45 to 85 per cent.

Van Ravesteyn<sup>518</sup> fed 150 mg. of copper sulfate per day for 3 days to adults. Approximately 65 to 75 per cent of the copper was recovered from the feces over a period of 6 to 9 days. The copper content of the bile was more than doubled but the excretion of copper in the urine was not affected. Following the intravenous injection of copper the blood level rose to 300 to 400 micrograms per cent and during the course of the next 2 to 4 hours gradually returned to normal. The excretion in the bile and urine rose temporarily and the fecal copper increased markedly. This increase was greater than could be accounted for by the excretion through the bile. Rabinowitch<sup>519</sup> has reported that the copper content of normal urine ranges from traces to 0.7 mg. per 24 hours. Tompsett<sup>513</sup> found 0.08 to 0.48 mg. per liter. Chou and Adolph<sup>471</sup> obtained an average value of 0.25 mg. per day and found that the excretion did not vary appreciably with changes in copper intake.

It has been estimated that the adult body contains between 100 and 150 mg. of copper.<sup>471</sup> During embryonic development copper becomes concentrated in the liver and reaches a maximum concentration at term.<sup>472, 520</sup> There is then a sharp decline after the second month.<sup>521</sup> The distribution of copper in human tissues has been reported in detail.<sup>471, 521-523</sup> The copper content of the liver has been noted to be increased in Mediterranean anemia,<sup>523, 524</sup> hemochromatosis,<sup>526</sup> cirrhosis of the liver,<sup>526, 527</sup> acute yellow atrophy of the liver,<sup>528</sup> tuberculosis,<sup>521</sup> and carcinoma.<sup>524, 525</sup> Sandberg, Gross, and Holly<sup>524, 529</sup> have made a study of the copper content of the liver, spleen, and stomach in a large series of cases of severe chronic disease accompanied by secondary anemia and have reported that there is a huge storage of copper in the depot organs. In cancer accompanied by anemia the marked increase in copper storage was out of proportion to the anemia and even took place in several cases in the absence of anemia. The retention was significantly higher in cases with extensive metastasis. Buchwald and Hudson<sup>530</sup> found that the copper content was high in the liver and bile, intermediate in the kidney, heart, and pancreas, and low in the tumor tissue and spleen in cases of malignancy.

There was little agreement in the earlier literature as to the amount of copper in the blood.<sup>305, 433, 496</sup> There was even less agreement as to the ratio of its distrib-

tion between the cells and serum. These disagreements were most likely the result of poor methods. It is now generally agreed that the range for whole blood copper in normal adult males is approximately 90 to 150 micrograms per cent while that for normal adult females is approximately 100 to 160 micrograms per cent.<sup>531-534</sup> Sachs, Levine, Hill, and Hughes<sup>532</sup> recently reported an average value of 102 micrograms per cent for whole blood copper in adult males and 107 micrograms per cent for adult females.<sup>532</sup> Normal females have been persistently found to have slightly higher values than males.

It is now generally agreed that copper is distributed approximately equally between the cells and serum.<sup>496, 531, 532, 534-536</sup> Sachs and his co-workers<sup>532, 535</sup> have reported serum values for normal males between 70 and 132 micrograms per cent and for normal females between 78 and 124 micrograms per cent. Cartwright, Jones, and Wintrobe<sup>536</sup> determined the copper content of the serum of 25 healthy adult males and 25 healthy adult females. The average value for the males was 116 micrograms per cent. The lowest value obtained was 92 and the highest 134 micrograms per cent. For the females the average was somewhat higher, 131 micrograms per cent with values ranging between 103 and 159 micrograms per cent. Nielsen<sup>537</sup> reports the serum copper for normal males as  $100 \pm 12 \gamma$  per cent and for normal females  $\pm 16 \gamma$  per cent.

Sachs and his group have made studies on whole blood copper as well as iron at various ages.<sup>532, 538, 539</sup> At birth there is an increase in iron and a decrease in copper. During the first 2 months of life there is a sharp and pronounced drop in iron and a sharp rise in copper. From 2 months until 1 year of age there is a gradual rise in both iron and copper. During the age period of 2 to 12 years the values for both iron and copper tend to remain stationary although the values for iron are lower than those found in adults and the values for copper are somewhat higher. At the onset of puberty the iron gradually increases until it settles in the adult range and the copper decreases until it reaches the normal. Thus from infancy throughout life there is an inverse relationship of the copper and iron of whole blood.

Many investigators have reported the presence of increased blood copper during pregnancy.<sup>422, 531, 533, 534, 540, 541</sup> In spite of this, blood taken from the umbilical cord has a low copper content.<sup>531</sup> In hypochromic microcytic anemia in infants and adults the whole blood copper is markedly diminished.<sup>422, 531</sup> The copper content of whole blood has been reported as increased in pernicious anemia,<sup>422, 531</sup> anemia of sepsis,<sup>534</sup> anemia associated with Addison's disease,<sup>542</sup> sickle cell anemia, Banti's disease, malaria, myelogenous leukemia, arsenic poisoning, and carcinoma.<sup>531</sup> In no case or condition has a hypocupremia been reported.<sup>532</sup>

In all of the conditions studied by Sachs and his group there has been an inverse relationship between the copper and iron content. As the iron falls the copper content tends to rise. Hypercupremia is the usual response to hypoferremia. This has been clearly illustrated in 2 cases of polycythemia vera<sup>531, 543</sup> treated with phenylhydrazine. As anemia developed the iron content of the blood diminished and the copper content increased. Then as polycythemia reappeared the iron rose and the copper fell to normal.

It must be pointed out that the above studies were done on whole blood. Since

blood iron is present principally in the hemoglobin molecule it would be expected that whenever anemia is present the total blood iron would diminish. It does not necessarily follow that there is a reciprocal relationship between serum iron and copper. Such studies would be most desirable. The iron content of the serum is known to be elevated in pernicious anemia. Sachs has reported that in this condition the whole blood copper is increased. This must mean that the copper content of red cells is greatly increased or that there is both a hypercupremia and hyperferremia in the serum. Simultaneous studies on cellular iron and copper and serum iron as well as copper in a variety of conditions, might uncover important facts pertaining to the metabolism of these two elements and to their relationship to erythropoiesis.

Elevated serum copper values have been reported in pregnancy<sup>532, 544</sup> and in infections accompanied by anemia.<sup>570, 544</sup> In both of these conditions there is a low serum iron. The serum copper content is subject to diurnal variations but is not affected by menstruation.<sup>544</sup>

5. *The function of copper.*—The precise manner in which copper is related to the formation of red cells is not understood. It is not a constituent of the hemoglobin molecule.<sup>545</sup> Since the administration of copper to anemic rats is followed by a rise in the erythrocyte count without a rise in hemoglobin it has been postulated that copper is essential for stroma formation of the cell or for the release of erythrocytes from the bone marrow rather than for hemoglobinogenesis.<sup>551, 546</sup> Schultz, Elvehjem, and Hart<sup>497</sup> state that it is not possible to assign to copper a specific function for formation of either erythrocytes or hemoglobin, as the two processes are interdependent.

When iron is fed to iron- and copper-deficient animals the total iron content of the liver and spleen increases in proportion to the amount of iron fed.<sup>547, 548, 549</sup> Thus in the absence of copper iron is absorbed and stored normally.<sup>549, 550</sup> If copper is then fed to these animals in place of iron, hemoglobin formation takes place and the iron content of the liver and tissues is reduced to a level of that found in severely anemic animals.<sup>547, 548</sup> From this it has been concluded, and generally accepted, that copper is essential for the mobilization of iron from the tissues and for its conversion into hemoglobin; or, stated in another manner, copper acts as a "catalyst" for the transformation of inorganic iron into hemoglobin.

In 1934 Cohen and Elvehjem<sup>551</sup> reported a marked decrease in the cytochrome A content of the heart, liver, and brain of rats with nutritional anemia. The feeding of small amounts of copper restored the cytochrome spectrum, while the feeding of iron was ineffective. With this lead, Schultz<sup>552, 553</sup> studied the cytochrome oxidase activity in the liver, heart, and bone marrow of copper-deficient rats and found that the activity of this enzyme was markedly diminished. Copper therapy initiated an immediate increase in cytochrome oxidase activity in the bone marrow. Maximal activity was approached within 24 hours. Iron, manganese, and cobalt did not affect the oxidase activity. Schultz and Kuiken have shown that the catalase activity of the liver, kidney, and blood of copper-deficient rats is markedly diminished.<sup>554</sup> When copper was given the catalase activity returned rapidly to normal.

Thus it has been shown that copper is essential for the activity of at least three enzymes. The common denominator between these three enzymes and hemoglobin

protoporphyrin type III no. 9 since it is a constituent of each of these compounds. The interesting question arises, Is copper essential for the formation of protoporphyrin? Schultze<sup>555</sup> attempted to answer this by determining whether or not protoporphyrin is excreted by anemic copper-deficient rats. He was able to isolate protoporphyrin type III no. 9 from the feces of such animals but unfortunately was not able to rule out the possibility of synthesis by intestinal flora. The question is, therefore, unanswered.

A second possibility is that copper is a structural component of cytochrome oxidase<sup>556, 557</sup> and catalase.<sup>558</sup> If this should be true the effects of copper on the blood would be due directly to its effect on cytochrome oxidase since it has been shown that a high activity of this enzyme in the bone marrow is intimately associated with hematopoiesis. It is unlikely, however, that these enzymes contain copper as structural component.<sup>559, 560</sup>

A third possibility suggested by Schultze<sup>553</sup> is that the effect of copper on hematopoiesis is dual, first on cytochrome oxidase activity of the bone marrow and second on the synthesis of hemoglobin.

There are numerous other recognized functions of copper. The oxidation of crystalline glutathione is accelerated in the presence of small amounts of copper<sup>561</sup> and it has been frequently observed that during recovery from hemorrhagic anemia the glutathione content of the blood is increased.<sup>562</sup> The catalytic effect of copper on the oxidation of cysteine<sup>563</sup> as well as ascorbic acid<sup>564</sup> has been reported. It has been stated that the addition of copper to a scorbutic diet prevents the appearance of signs of scurvy in guinea pigs and causes their recession when incorporated after the appearance of scurvy.<sup>565, 566</sup> Glycolysis is known to be activated by copper<sup>564</sup> and accelerated in anemia. What relationship, if any, these functions may have to the formation of red cells is now only speculative.

C. Cobalt.—The role of cobalt in erythropoiesis is unique. A deficiency results in anemia. The administration of small amounts to normal animals produces erythrocytosis, whereas the administration of large amounts depresses erythropoiesis.

The enzootic occurrence of cobalt deficiency has been reported from several regions of the world. The soil and herbage in these areas have been shown to be deficient in cobalt. Anemia is present, oftentimes severe, but its morphological characteristics have not been carefully studied. The disease caused by a deficiency of cobalt is known by various names in different parts of the world.

Enzootic marasmus is a disease of cattle and sheep occurring in a localized area of about 5000 acres in the Denmark district near the southeastern coast of Western Australia.<sup>567</sup> Clinically the disease is characterized by progressive emaciation, weakness, a rough coat, and pallor of the mucous membranes. The animals develop a craving for harsh fibrous brush and in the late stages anorexia is pronounced. Diarrhea is often seen in young calves. In cows lactation is diminished, oestrus rarely occurs, and breeding is seldom successful. Abortions are common. Death occurs in from 6 weeks to 2 years after symptoms are first noticed, the shorter duration being usually observed in the young animals. Pathologically the most significant finding is hemosiderosis of the spleen, liver, and kidneys.<sup>568</sup> Fatty infiltrations in the liver are frequently seen. Blood studies reveal a marked anemia which in

lambs is normocytic hypochromic and in calves is either normocytic or slightly microcytic and hypochromic. The reticulocytes are reduced. Anisocytosis and poikilocytosis are common. There is a hypoplasia of erythrocytic tissue in the bone marrow. A megaloblastic or erythroblastic bone marrow has not been reported. There is no evidence of increased blood destruction. The administration of 0.1 mg. of cobalt daily to sheep or a dose of 0.3 to 1.0 mg. daily to cattle is capable of both preventing and curing the disease.<sup>569, 570</sup> Animals so treated have been maintained in good health for periods up to 12 months while still grazing on affected land. The administration of copper has no beneficial effect although the copper stores are often low.<sup>571</sup> Low blood copper values have not been found. Following the administration of cobalt the hemoglobin rises initially and then falls sharply. After this the blood returns gradually to normal. It has been suggested that the temporary fall in hemoglobin is due to the fact that the iron stores, even though excessive, are rapidly used and finally depleted and that the hematopoietic system is temporarily unable to support the strain placed on it by excessive growth. The hemosiderosis has been observed to disappear during cobalt therapy. Reticulocytosis following therapy has not been observed. Traces of nickel have been found to increase the action of suboptimal doses of cobalt. Liver cures as well as prevents the disease and its action is not thought to be due to its cobalt content since this is low. It has been suggested that the potency of liver may be due to the presence of a stored factor and that cobalt may function through the production of this factor in the body.

A disease similar to enzootic marasmus has been observed in sheep grazing in certain coastal regions in Southern Australia.<sup>572-574</sup> This has been termed "coast disease" and is characterized clinically by listlessness, lethargy, anorexia, weakness, pallor, and finally death. Wool growth is affected and the fleece is dull. Edema is oftentimes present. At autopsy marked hemosiderosis of the pancreas and liver and to a lesser extent the spleen is observed. The total blood volume appears to be reduced although determinations have not been made. A severe anemia is present. It is stated that the anemia is microcytic and normochromic. Blood smears show anisocytosis, some polychromatophilia, and an occasional nucleated red blood cell. Treatment with copper is ineffective. Treatment with cobalt alone permits growth but fails to restore the hemoglobin entirely to normal. Treatment with both copper and cobalt prevents the disease and once the disease is manifest restores the animals rapidly to normal. The average hemoglobin content in volumes per cent of oxygen of untreated animals was 2.4, in copper-treated animals 4.7, in animals treated with cobalt 9.9, and in animals treated with copper and cobalt 13.8. In an additional group of animals treated with iron, nickel, manganese, and zinc in addition to copper and cobalt the final hemoglobin value was the same as in the group treated with only cobalt and copper. Copper analyses revealed that the content of the tissue was reduced although there was no significant alteration from normal in the amount of copper in the blood. It would appear that "coast disease" is due in most cases to a deficiency of both cobalt and copper but the occurrence of uncomplicated cobalt deficiency in sheep in Southern Australia has been noted by McDonald.<sup>575</sup> Hematological examination in these animals revealed a marked variability in the degree of anemia present. Some animals even when moribund

showed almost normal blood hemoglobin levels, whereas in others the hemoglobin was extremely low. The anemia was normocytic and blood smears showed only slight poikilocytosis. General observations at postmortem suggested that one of the most striking features of the anemia was a gross reduction in blood volume, but no direct measurements were made.

A disease of sheep similar to "enzootic marasmus" and "coast disease" known as "bush sickness" or "Morton Mains disease" has been reported from New Zealand.<sup>576-582</sup> This condition likewise responds to cobalt therapy. Anemia exists but has not been studied. A similar condition has been reported as occurring in sheep in Canada.<sup>583, 584</sup> A disease of sheep known as "pine disease," "pining," "border pine," "Cheviot pine," or "Northumbrian pine" occurring in Scotland, characterized by emaciation, lethargy, retardation of growth, unthriftiness, anemia, and a fatal termination, has been reported.<sup>585, 586</sup> The effect of cobalt on this condition is in dispute.<sup>587-590</sup>

Cobalt deficiency in cattle has been reported as occurring in certain regions of the United States. Geyer, Rupel, and Hart<sup>591</sup> have reported a condition in cattle in the northeastern region of Wisconsin characterized by unthriftiness and anemia, which responds to 3 mg. of cobalt per animal per day. A condition known as "Grand Traverse disease" or "Lake Shore disease" and characterized by unthriftiness, anorexia, depraved appetite, emaciation, anemia, and finally death has been observed in Michigan.<sup>592</sup> Following the administration of cobalt the animals exhibit a spectacular return of appetite and a progressive improvement in their condition. The hemoglobin shows an initial decrease of from 10 to 20 per cent and is then followed by a gradual return to normal. A disease in Florida cattle known as "hill sick" or "salt sick" has been reported by Neal and Ahmann.<sup>593</sup> The affected animals showed a rough coat, scaliness of the skin, listlessness, retarded sexual characteristics, anorexia, emaciation, and muscular atrophy. Anemia, microcytic and hypochromic in type, accompanied by a reduction in reticulocytes, anisocytosis, poikilocytosis, and a lymphocytosis, was observed. Pathologically myocardial degeneration, a decrease in splenic pulp, liver degeneration, and hemosiderosis of the liver and spleen were found. The condition was found to be aggravated by iron and copper but responded rapidly to cobalt therapy.

An experimental anemia due to cobalt deficiency has not been produced in rats and dogs. Underwood and Elvehjem<sup>594</sup> were unable to demonstrate a cobalt deficiency in rats on a milk diet. Frost, Elvehjem, and Hart<sup>595</sup> have reported that in most dogs the addition of iron and copper to milk results in normal hemoglobin building. Small amounts of cobalt in addition to iron and copper therapy stimulated hematopoiesis in certain dogs in which the rate of blood formation appeared unusually slow. In a later paper from the same laboratory rapid increases in hemoglobin, volume of packed red cells and red cell counts to normal values were noted in all animals when iron and copper together were fed to dogs maintained on milk and rendered anemic by bleeding.<sup>450</sup>

Studies in man are too few to warrant conclusions.<sup>596-599</sup> In general, as one would expect, cobalt has not been found to be of value in the treatment of anemias. Whether or not there is a human requirement for cobalt cannot be stated. It may be

significant that iron compounds used therapeutically contain small amounts of cobalt.<sup>600</sup>

The administration of small amounts of cobalt to normal rats,<sup>596, 601-606</sup> dogs,<sup>607-609</sup> guinea pigs,<sup>610</sup> frogs,<sup>610</sup> mice,<sup>610</sup> rabbits,<sup>596, 611-613</sup> chickens,<sup>303</sup> pigs,<sup>613</sup> and ducks<sup>614</sup> produces a definite and marked polycythemia which is accompanied by a reticulocytosis, hyperplasia of the bone marrow and an increased erythropoietic activity of the spleen and liver. Following the subcutaneous injection of cobalt into rabbits there is a marked reticulocytosis, normoblasts appear in the circulating blood, there is anisocytosis and polychromasia of the red cells, an eosinophilia and transient lymphocytosis.<sup>612</sup> The polycythemia is a true one and is not due to a decreased blood volume.<sup>615</sup> The mechanism by which cobalt produces such a polycythemia is not understood.<sup>616</sup> The respiratory activity of the bone marrow is not impaired.<sup>616</sup> Furthermore, such a polycythemia increased work performance under conditions of reduced oxygen tension.<sup>617</sup> Cobalt does not produce polycythemia in splenectomized rats<sup>602</sup> or when the diet is deficient in iron or in copper.<sup>44</sup> However, cobalt produces a polycythemia in low-protein rats at approximately the same rate and to about the same degree as in normal rats.<sup>618</sup> Ascorbic acid,<sup>611</sup> manganese,<sup>619</sup> concentrated liver extract,<sup>601</sup> ventriculin,<sup>601</sup> whole beef,<sup>603</sup> and whole liver<sup>603</sup> have all been reported to counteract the effects of cobalt. Frost, Spitzer, Elvehjem, and Hart<sup>620</sup> observed an inhibition of the normal hematopoietic response to iron and copper feedings in dogs made anemic by hemorrhage and fed cobalt prior to the addition of iron and copper. Hematopoietic activity was resumed on the feeding of whole dry liver or liver extract. It is of interest that large doses of cobalt depress erythropoiesis.<sup>508, 614, 620</sup>

Studies on the metabolism of cobalt in rats have been made by Greenberg and his group using the radioactive isotope.<sup>424, 621</sup> Within 72 hours following the injection of 0.1 mg. of the isotope 3.5 per cent was excreted in the bile, approximately 65 per cent in the urine, and 5 per cent in the feces. Two and one-half per cent was demonstrable in the liver. During the same interval of time following the oral administration of a similar dose 2 per cent was excreted in the bile, 20 per cent in the urine, and 40 per cent in the feces. Three and one-half per cent was demonstrable in the liver. Small amounts were also found in other organs including the bone marrow. After 4 days 95 per cent of the radio-cobalt given by either route had been excreted. These results are in accord with the work of others in animals<sup>622, 623</sup> and are in sharp contrast to iron since only 2 to 8 per cent of this element when given parenterally is excreted in a comparable period. Evidently the body retains very little cobalt and the requirement must be extremely small. The bodies of rats on a normal diet contain only about 5 micrograms of cobalt.<sup>624</sup>

In human beings Penati and Ruata<sup>625</sup> found that when cobalt was given orally only 3.5 per cent appeared in the urine in 24 hours. Le Goff<sup>626</sup> injected 24 mg. of cobalt chloride intramuscularly into a man and recovered 28 per cent of the salt in the urine within the next 18 hours. Kent and McCance<sup>627</sup> after the intravenous injection of cobalt into a man found that 22 per cent was excreted in 1 week. Seventy-four per cent of this amount was excreted by the kidneys. They concluded

that in man once cobalt reached the tissues, the process of elimination was very slow.

## V. DISCUSSION

It is the ultimate goal of one of the phases of research in hematology to write the precise chemical reactions, step by step, for the formation of the red cell. It would be desirable in this review to follow the description of the factors concerned in erythropoiesis by a complete discussion of their interrelationships and of the manner in which they are concerned with erythropoiesis. Unfortunately, with the knowledge now available this is not possible with any degree of completeness or accuracy.

The factors concerned in erythropoiesis might be classified in several ways. In this review a classification based on the chemical nature of the substances has been used. Another classification might be as follows:

- I. Formation of the red cell stroma
  - A. Substances used for the construction of the stroma
  - B. Substances essential for the formation of the stroma but which are not included in the stroma
- II. Formation of the hemoglobin molecule
  - A. Substances used in the construction of hemoglobin
  - B. Substances essential for the construction of hemoglobin but which are not included in the molecule

The factors known to be used for the construction of red cells are presented schematically in figure 2.

The dried substance of the stroma is composed of protein (40 to 60 per cent), lipids (10 to 12 per cent),<sup>628</sup> inorganic salts, and enzymes. The amino acids include arginine, lysine, histidine, tyrosine, tryptophan, cystine, and methionine. The lipid fraction<sup>629</sup> is chiefly in the form of phospholipids (cephalin, sphingomyelin, lecithin) (58 to 72 per cent) but in addition there are free cholesterol and cholesterol esters as well as neutral fat. Adenine-ribose-nucleotide,<sup>21</sup> glyoxalase,<sup>630</sup> and certainly other enzymes are present. The various mineral elements present include sodium, potassium, magnesium, phosphorus, copper, and probably calcium as well as others.

Although copper is a constituent of the stroma its function is not known. It has been suggested that copper is essential for the mobilization of iron from the tissues and for its conversion into hemoglobin. It is true that when copper is administered to a copper-deficient animal iron is mobilized. This would be true, however, simply because erythropoiesis proceeds. A direct relation between iron and copper metabolism need not be implied.

The constituents of hemoglobin are heme and globin. Heme consists of the union of iron and protoporphyrin. The protoporphyrin is attached to the globin molecule through the two carboxyl (propionyl) groups. Very little is known concerning the precursors of protoporphyrin. As far as is known the animal does not depend upon a dietary supply of pyrrole or its derivatives. It is generally assumed, although

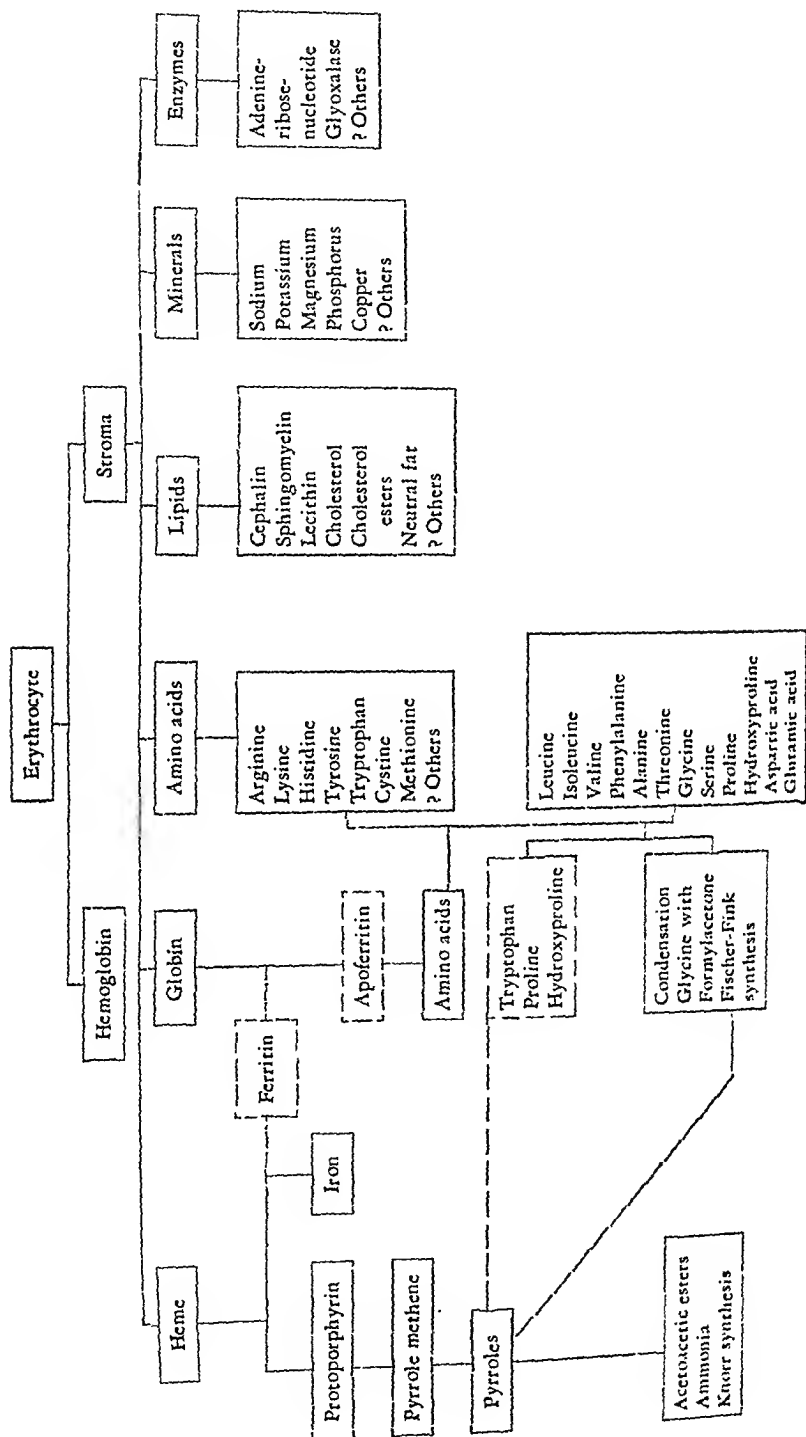


FIGURE 2. A SCHEMATIC DIAGRAM OF ERYTHROPOIESIS

TABLE 13.—Summary of the Hematologic Manifestations of the Various Deficiency Syndromes

Substance	Species	Type of Anemia	Bone Marrow	Leukopenia	Thrombopenia	Reticulocytosis	Serum Iron	Hemosiderosis	Neurological Lesions
Riboflavin	Rat, dog, pig, monkey	Normocytic		±	0		N		
Nicotinic acid	Dog	Normocytic or macrocytic Normochromic	Hypoplastic	0		0		+	0
Pyridoxine	Chick, rat, dog, pig	Microcytic, hypochromic	Hypoplastic	0	0	+	H	+	+
<i>Lactobacillus casei</i> factor	Rat	Normocytic, (?) normochromic	Variable	+	+				0
<i>Lactobacillus casei</i> factor	Man (pernicious anemia)	Macrocytic, normochromic	Megaloblastic	+	+	+	H	+	+
Vitamin B <sub>6</sub>	Chick	Macrocytic, hypochromic Variable		+	+				0
Vitamin M	Monkey	Normocytic		+	+				0
Extrinsic factor	Man	Macrocytic, normochromic	Megaloblastic	+	+	+	N, L		0
Pantothenic acid	Pig, (?) rat	Normocytic, normochromic		0	0		N	0	+
Tryptophan	Rat, pig	Normocytic, normochromic	Normoplastic or hypoplastic	±	0	0	N	0	0
Lysine	Rat	(?) Macrocytic						0	0
Phenylalanine	Rat							0	0
Isoleucine	Rat							0	0
Iron	Many species including man	Microcytic, hypochromic	Hyperplastic Normoblastic	0	0	+	L	0	0
Copper	Chicken, sheep, cattle, rabbit, rat, dog, pig, mice, (?) man	Microcytic, hypochromic		0	0	+		+	+
Cobalt	Cattle, sheep	Normocytic or microcytic Usually hypochromic	Hypoplastic	0	0	0		+	0

+, present; 0, absent; N, normal; H, high; L, low.

there is not a great amount of evidence, that protoporphyrin is an intermediate in the synthesis of hemoglobin and that substituted pyrroles are intermediates in the synthesis of protoporphyrin. It was through these intermediates that it was possible for Fischer<sup>1</sup> to synthesize heme *in vitro*. That such steps take place *in vivo* is

only speculation. It is true that protoporphyrin is normally present in erythrocytes and especially in young red cells and is present in relatively large amounts in the developing chick embryo.

Recently, the identities of two precursors of protoporphyrin, a derivative of acetic acid and glycine have been established.<sup>297, 631</sup> Since condensations involving acetoacetic esters and ammonia are known to yield pyrroles *in vitro* (Knorr synthesis)<sup>632</sup> and since Fischer and Fink<sup>633</sup> have found that pyrroles are formed by condensing formylacetone with glycine the new *in vivo* findings are exceedingly interesting. Thus the precursors of protoporphyrin may be very simple substances and this may help to explain the ease with which the body can synthesize protoporphyrin, why the body can afford to discard the bile pigments rather than conserve them, and why anemia due to a failure to synthesize protoporphyrin is difficult to produce. The possibility must be kept in mind, however, that the hemoglobin molecule may be constructed around the iron and that true porphyrins and pyrroles are not present at any time during the synthesis except in small quantities produced in a side reaction. It has been suggested that the pyrrole rings of tryptophan, proline, and hydroxyproline might be utilized in the synthesis of protoporphyrin (figure 2). Direct evidence for this is completely lacking.

Globin is undoubtedly formed from various combinations of amino acids. Very little is known of the steps of this synthesis, of the nature of the intermediate compounds, or of the site or sites at which the synthesis takes place in the body. It is interesting, and may or may not be significant, that extensive bleeding in horses lowers the apoferritin content of the spleen. This suggests that this substance may be utilized directly for hemoglobin synthesis. It is even possible, although there is no evidence at present to demonstrate it, that ferritin as such may be utilized in hemoglobin synthesis.

The hematological manifestations of the various known deficiency syndromes are summarized in table 13. These syndromes have been inadequately studied. In each of the experimental anemias it would be desirable to have data, for example, concerning the erythrocyte protoporphyrin, porphyrin excretion, serum copper, serum cobalt, various bone marrow enzymes, and so forth. As such information is obtained it may be expected that the relationships between the various factors will be elucidated.

## VI. SUMMARY

Riboflavin is essential for normal erythropoiesis in rats, dogs, pigs, and monkeys. There is no evidence that this vitamin is required for normal erythropoiesis in man. The anemia in swine is normocytic.

Nicotinic acid deficiency is accompanied by a severe anemia in dogs. The type of anemia produced is normochromic and may be either macrocytic or normocytic and is associated with a mild reticulocytosis. Limited observations indicate that the bone marrow is hypoplastic and that erythropoiesis stops at the erythroblastic level. An anemia due to a deficiency of this vitamin has not been demonstrated in other species nor in man.

Pyridoxine is essential for normal erythropoiesis in chicks, rats, dogs, and pigs.

The anemia is microcytic and slightly hypochromic in type. Anisocytosis, microcytosis, polychromatophilia, and normoblasts can be seen in the blood smear. An irregular reticulocytosis is present. The bone marrow is hyperplastic and there is an increase in the nucleated red blood cells. The anemia is accompanied by hemosiderosis of the tissues, an elevated serum iron level, and degeneration in the nervous system. There is no evidence of an increased rate of hemolysis. No relationship between pyridoxine and erythropoiesis has been demonstrated in man.

The "*Lactobacillus casei* group" includes the norite eluate factor, the *L. casei* factor from liver, folic acid, the *Streptococcus lactis* R factor of Keřesztesy et al., the yeast factor of Stokstad, the factor of Hutchings et al., vitamin M<sub>11</sub>, xanthopterin, vitamin B<sub>6</sub>, vitamin B<sub>6</sub> conjugate, vitamins B<sub>10</sub> and B<sub>11</sub>, and pyracin.

The *L. casei* factor from liver has been identified as pteroylglutamic acid. The available evidence indicates that the norite eluate factor, folic acid, vitamin M, vitamin B<sub>6</sub>, vitamin B<sub>10</sub>, and vitamin B<sub>11</sub> are identical with pteroylglutamic acid. The *Streptococcus lactis* R factor of Keřesztesy et al. may be pteroic acid. The yeast factor of Stokstad is unidentified. The fermentation factor of Hutchings et al. has been identified as pteroyltriglutamic acid. Vitamin B<sub>6</sub> conjugate is now known to be pteroylheptaglutamic acid. Thus the various members of this group are closely related chemically and represent minor alterations of a basic structure. The corresponding deficiency syndromes are probably identical. In the rat the deficiency is manifested by severe normocytic anemia, severe granulocytopenia, leukopenia, and thrombocytopenia. Nucleated red cells appear in the peripheral blood. Bone marrow studies suggest a maturation arrest in the early stage of development of all three of the cellular elements of the blood. The manifestations of the deficiency in the chick are macrocytic anemia, leukopenia, and thrombocytopenia. Again immature red cells are present in the peripheral blood. In the monkey the manifestations of the deficiency are normocytic anemia, leukopenia, and thrombocytopenia. In human beings the synthetic *L. casei* factor from liver (pteroylglutamic acid) has been shown to be effective in the treatment of various types of macrocytic anemia including pernicious anemia and sprue. The relation of this substance to the anti-pernicious anemia substance in liver remains to be determined.

The extrinsic factor of Castle is still unidentified. It now seems reasonable that it is related in some way to pteroylglutamic acid. It is unlikely that it is identical since the synthetic *L. casei* factor is effective even in the absence of normal gastric juice. A deficiency of the extrinsic factor in man results in an anemia which is identical with pernicious anemia and the bone marrow is cytologically indistinguishable. An accompanying neutropenia and thrombocytopenia are also frequently seen. The anemia responds rapidly to the parenteral administration of highly purified antipernicious anemia liver extracts and to pteroylglutamic (folic) acid. Achlorhydria is generally not present. Macrocytic anemia of nutritional origin occurring in the tropics varies from this anemia in one important aspect. It fails to respond to highly purified liver extracts. This strongly suggests that the factor responsible for the deficiency is distinct from that of the extrinsic factor of Castle. A deficiency of this factor has been produced in monkeys and the deficiency syndrome consists of a macrocytic anemia with a megaloblastic bone marrow. The

anemia fails to respond to highly purified liver extracts which are effective in the treatment of pernicious anemia but does respond to crude liver extracts and to marmite, an autolyzed yeast extract. The relation between this factor and the *L. casei* factor has not been investigated.

The role of ascorbic acid in erythropoiesis is not clear. Although the scorbutic state in both guinea pigs and human beings is frequently accompanied by anemia it is questionable whether the anemia is due specifically to a deficiency of ascorbic acid. Much of the animal experimentation is inconclusive because pure ascorbic acid supplements were not used. Further work in animals is needed. In man it has been both asserted and denied that synthetic ascorbic acid is effective in relieving the anemia. It would seem, however, that there are some scorbutic patients who respond specifically to pure ascorbic acid. The anemia accompanying scurvy has been reported as macrocytic, normocytic, and microcytic. An induced, uncomplicated ascorbic acid deficiency in a human being did not result in anemia.

Pantothenic acid deficiency results in a normocytic anemia of moderate degree in pigs in about two-thirds of the animals. There is evidence which suggests that a deficiency of this vitamin in rats may result in anemia, granulocytopenia, and bone marrow hypoplasia. Not all animals show these changes and pantothenic acid, although completely preventive, does not exert a curative action in all animals. There seems to be a relation between pantothenic acid deficiency and a deficiency of the *L. casei* factor in the rat.

Choline deficiency in dogs results in a severe anemia. In many animals this change is irreversible. This may be explained by the irreversible liver damage which is present.

Biotin is necessary for the production of hemoglobin values greater than 14 grams per cent in dogs maintained on a highly purified ration. There is no evidence that biotin has an effect on erythropoiesis in other species.

In addition to the factors described above it has been shown that monkeys, pigeons, and guinea pigs require at least one more additional factor for normal erythropoiesis.

There is no evidence that thiamine, p-aminobenzoic acid, and inositol are concerned in erythropoiesis in any species.

Considering the relative size of the globin fraction of the hemoglobin molecule it is understandable that a deficiency of protein results in anemia. This has been demonstrated in rats and dogs. It has been pointed out that because of a marked reduction in the total blood volume only when the total circulating hemoglobin is determined and adjusted to a unit of surface can the true severity of the anemia be appreciated. Equine globin contains all ten of the "essential" amino acids and at least nine "nonessential" amino acids. Human globin has not been so extensively studied. It would be expected that a deficiency of any one of the "essential" amino acids would give rise to anemia. Actually, specific deficiencies of tryptophan, lysine, phenylalanine, and isoleucine have been produced in the rat and anemia developed in each instance. The morphological characteristics of these anemias have not been carefully investigated. The anemia due to tryptophan deficiency in the rat has been stated to be normocytic and normochromic. An anemia probably

due to a lack of tryptophan has been produced in pigs. This anemia is normocytic, normochromic, and accompanied by a hypoplastic or normoplastic bone marrow and a normal level of iron in the serum. No increase of hemosiderin in the tissues has been noted. Whether the anemia produced in rats by feeding deaminized casein is due to a toxic substance rather than a deficiency of lysine is unsettled although large amounts of lysine prevent its development. Evidence that glycine is utilized in the synthesis of the pyrrole rings of protoporphyrin has been obtained by labeling this amino acid with N<sup>15</sup> and feeding the labeled compound to rats. Pyrroles have also been synthesized *in vitro* from glycine. Similar evidence is available to indicate that acetic acid, or a derivative of it, is utilized for porphyrin synthesis.

Three mineral elements, iron, copper, and cobalt, have been shown to be essential for normal erythropoiesis in at least one species each. Iron is probably required for erythropoiesis in all mammals. A deficiency results, at least in the chronic stages, in a microcytic hypochromic anemia and is accompanied by a normoblastic, hyperplastic bone marrow and a low serum iron level, an increased amount of protoporphyrin in the erythrocytes, and an elevated serum copper level. Nucleated red blood cells are occasionally seen in the peripheral blood and the reticulocytes are increased.

The fundamental concepts of iron metabolism have changed greatly in recent years. These may be summarized. Iron is absorbed chiefly in the duodenum. In man it is absorbed principally as ferrous iron. Dogs absorb both valence forms well although some animals absorb the ferrous form more readily than the ferric form. Rats absorb both forms equally well. The absorption of iron is also dependent upon the concentration of the iron in the intestine, upon the solubility of the iron salt, and in the human being at least upon the presence of reducing substances in the diet as well as the reducing action of the gastric hydrochloric acid. In addition to these factors the need of the body for iron may determine, to a certain degree, the amount absorbed. This is known as the "selective absorption" theory. Recently it has been suggested that apoferritin acts as a receptor compound in the mucosal cell. As the concentration of the plasma iron falls, ferrous iron is removed from the mucosal cell resulting in a diminution of ferritin in the mucosa. When the ferritin has diminished to a point where the cell is no longer saturated with respect to ferrous iron, more iron is absorbed into the mucosal cell. Once absorbed the iron is transported in the plasma to the tissues where it is stored to a great extent as ferritin, a protein-iron complex. The iron is then used over and over again for hemoglobin synthesis. Iron is excreted from the body in only insignificant quantities. This theory requires substantiation.

Copper has been shown to be essential for normal erythropoiesis in chickens, mice, rats, rabbits, dogs, pigs, sheep, cattle, and infants. A deficiency of this mineral in rats is manifested by a microcytic hypochromic anemia and a moderate reticulocytosis. A condition due to a deficiency of copper, known as "enzootic ataxia," occurs in sheep in Western Australia. Anemia may be severe. In young lambs it is microcytic and hypochromic and is accompanied by demyelination of the nervous system and hemosiderosis of the tissues. In adult sheep the anemia is slightly macrocytic and hypochromic. Blood smears reveal anisocytosis, poiki-

locytosis, Howell-Jolly bodies, normoblasts, numerous macrocytes, stippling, and polychromatophilia. Similar blood changes have been reported in copper-deficient cattle in Western Australia. In nutritional anemia in infants the rate of erythropoiesis is accelerated when copper is given in addition to iron. In adults supplemental copper therapy may be of value in a few cases. Such cases, if they occur, are rare. Most cases will respond if adequate doses of iron are given. This does not necessarily indicate that copper is not needed for erythropoiesis or that it is not a dietary essential but rather that the quantities needed are so small that sufficient copper is present in the body stores in adult life, in the diet, or as a contaminant in the iron used therapeutically to supply the needs. No case of uncomplicated copper deficiency has been reported in man. The manner in which copper is related to the formation of red cells is not understood.

The role of cobalt in erythropoiesis is unique. A deficiency results in anemia. The administration of small amounts to normal animals produces a polycythemia, whereas the administration of large amounts depresses erythropoiesis. The enzootic occurrence of cobalt deficiency in sheep and cattle has been reported from various regions of the world. Anemia is present and is oftentimes severe. The anemia is either normocytic or microcytic and usually hypochromic. Blood smears reveal anisocytosis and poikilocytosis. There is a hypoplasia of erythrocytic tissue in the bone marrow, hemosiderosis of the tissues and a reduction in reticulocytes in the blood. An experimental anemia due to cobalt deficiency has not been produced in either rats or dogs. There is no substantial or convincing evidence that cobalt is needed by human beings for normal erythropoiesis. The administration of small amounts of cobalt to normal rats, dogs, guinea pigs, frogs, mice, rabbits, chickens, pigs, and ducks produces a marked polycythemia which is accompanied by a reticulocytosis, hyperplasia of the bone marrow, and an increased erythropoietic activity in the spleen and liver. Larger doses of cobalt inhibit erythropoiesis. The metabolism of cobalt is unlike that of iron. The excretion of cobalt from the body once it is absorbed is exceedingly rapid and is principally through the kidneys.

In conclusion, certain vitamins, namely, riboflavin, nicotinic acid, pyridoxine, "folic acid," and the extrinsic factor, have been shown to be essential for normal erythropoiesis in at least one species each. It has been claimed that ascorbic acid, pantothenic acid, choline, and biotin play a role in erythropoiesis but these claims need substantiation. There is no substantial evidence that thiamine or inositol is concerned in red cell formation. The significance of p-aminobenzoic acid has yet to be determined. Protein is essential for normal red blood cell formation. The globin fraction of the hemoglobin molecule contains all ten of the "essential" amino acids as well as many of the "nonessential" ones. The stroma of the red cells also contains amino acids. It is logical, therefore, to assume that in the absence of any one of the so-called essential amino acids hemoglobin formation cannot take place normally. Actually specific deficiencies of tryptophan, lysine, phenylalanine, and isoleucine have been produced in the rat and anemia has developed in each instance. There is evidence to show that glycine and acetic acid, or a derivative of it, are utilized in the synthesis of the pyrrole rings of protoporphyrin. Three mineral elements, iron, copper, and cobalt, have been shown to be essential for normal erythropoiesis.

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## EDITORIAL

### THE MAMMALIAN RED CORPUSCLE

ONE of the most highly specialized structures in the mammalian organism is the red corpuscle. Its functional activity is carried on after the nucleus has been lost and its efficiency is so high that it consumes almost none of the gas which it transports.

This unique structure is peculiarly susceptible to study, since maturing cells may be observed in material aspirated from the bone marrow and the fully developed corpuscles are readily obtainable in any quantity. Approximately nine tenths of the red corpuscle (dry weight) is made up of hemoglobin and the remainder is stroma. The latter is composed of proteins and lipids, whereas hemoglobin represents a combination of protoporphyrin with iron and globin. Morphological studies have indicated how the red corpuscle develops from a nucleated stage. By the use of simple staining methods the formation of hemoglobin within the red cell can be observed. As compared with other proteins, hemoglobin is one of the most easily purified proteins, and consequently it has been the subject of extensive study.

Such promising advantages from the standpoint of research represent a challenge to the investigator. It is of significance, therefore, that as yet the chemical steps in the formation of hemoglobin and of stroma are quite obscure. Investigation, especially in recent years, has afforded some insight into iron metabolism, although many questions remain to be solved. More needs to be learned about the factors which influence and control the absorption of iron, the manner in which it is held in the tissues and the circumstances under which it is released and utilized. The globin of the hemoglobin molecule, it is assumed, is derived from the metabolic pool of protein. With the aid of heavy isotopes it has been shown that glycine and acetic acid are precursors of protoporphyrin. The steps in its synthesis and the nature of other precursors need to be elucidated.

As to the manner in which the union of iron, protoporphyrin, and globin is brought about, little indeed is known. It seems that copper plays some part, but its mode and site of action are obscure. Little more is known about cobalt than the fact that in its absence anemia can develop and that in certain species its administration leads to polycythemia. With the isolation of various components of the B complex it has been possible to learn that certain dietary essentials are necessary for normal erythropoiesis. It is quite clear that, in certain species at least, the lack of pyridoxine leads to a profound metabolic disturbance which is accompanied by anemia, an alteration in iron metabolism and a fault in the handling of tryptophan. There is evidence, also, that riboflavin and niacin are concerned in hematopoiesis. The discovery of the effectiveness of pteroylglutamic acid ("folic acid") in relieving the macrocytic anemia associated with pernicious anemia and sprue, led to the expectation that the nature of the extrinsic factor and the antipernicious anemia principle would be discovered momentarily. In-

stead we have learned about conjugates, conjugases, and inhibitors, with the result that there is, at the moment, more confusion than light. It is to be hoped that this represents the storm before the calm and that in the near future a clear picture of one or more of the steps entailed in the synthesis of hemoglobin will be developed.

MAXWELL M. WINTROBE, M.D.

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# NEWS AND VIEWS

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The following letter has been received from Dr. S. J. Thannhauser of Boston.

To the Editor:

Dear Sir:

S. Estren, M.D., John F. Suess, M.D., and William Dameshek, M.D., published under the title, "Congenital Hypoplastic Anemia Associated with Multiple Developmental Defects (Fanconi Syndrome)," a case report of a girl with hypoplastic anemia in association with other congenital defects. G. Fanconi reported for the first time, in 1927, "Familial Hypoplastic Anemia." Since then several authors, as quoted by S. Estren, et al., referred to this syndrome as "Fanconi Syndrome." G. Fanconi also described in several papers<sup>1</sup> an entirely different syndrome comprising tubular kidney dysfunction, hypophosphatemia, renal glycosuria, rickets and disturbed metabolism of amino acids. Sturzenegger<sup>2</sup> as well as McCune<sup>3</sup> and co-workers, and recently Fuller Albright<sup>4</sup> referred to this remarkable syndrome as "Fanconi Syndrome."

In attaching the name of Fanconi to two entirely different syndromes first described by this author, there is apt to be confusion. I would suggest to the authors who study the etiology of these different syndromes to designate the syndrome in a different way but certainly without curtailing the authorship of G. Fanconi.

Yours very sincerely,

S. J. Thannhauser, M.D.

Dr. S. Estren, 1489 East 8th Street, Brooklyn, New York, replies as follows:

Dear Doctor Thannhauser:

At the time the paper in question was in preparation it was known that Dr. Fanconi had described at least two and possibly three separate syndromes to which his name has since become attached. One of these was the congenital hypoplastic anemia syndrome; another, that of "renal rickets." I have been unable to find others, although I believe that there is at least one other in the literature. Certainly the indiscriminate use of the eponym "Fanconi" for entirely unrelated disorders is potentially confusing, although in actual practice the very knowledge of the existence of two separate disorders probably has

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<sup>1</sup> FANCONI, G.: (a) Die nicht diabetischen Glykosurien und Hyperglykämien des älteren Kindes. *Jahrb. f. Kinderh.*, 133: 257, 1931; (b) Der nephrotischglykosurische Zwergwuchs mit hypophosphatämischer Rachitis. *Deutsches med. Wchnschr.*, 62: 1169, July 17, 1936; (c) Der frühinfantile nephrotisch glykosurische Zwergwuchs mit hypophosphatämischer Rachitis. *Jahrb. f. Kinderh.*, 147: 299, 1936.

<sup>2</sup> STURZENEGGER, H.: Zur pathologischen Anatomie des frühinfantilen nephrotischglykosurischen Zwergwuchses mit hypophosphatämischer Rachitis (Fanconi). *Ann. Paediat.*, 153: 1, 1939.

<sup>3</sup> McCUNE, D. J., MASON, H. H., AND CLARKE, H. T.: Intractable Hypophosphatemic Rickets with Renal Glycosuria and Acidosis (The Fanconi Syndrome). *Am. J. Dis. Child.*, 65: 1943.

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obviated such confusion. (A parallel problem is perhaps to be noted in the two diseases described by Recklinghausen and generally considered, at least until recently, to be unrelated.)

Personally, I think your suggestion is certainly a valid one, but cannot suggest a simple term which would be as descriptive of the disorders as the ambiguous term "Fanconi." The respective "Fanconi's anemia" and "Fanconi's renal rickets," although less equivocal, are hardly more descriptive. Probably the best solution is to use the lengthier terminology (as in the title of our article) and the eponym "Fanconi." This is one case in which, I believe, an eponymic designation is desirable in describing a disorder.

Sincerely yours,

S. Esterl, M.D.

The editor has received the following letter from Dr. J. Alexandrow Cracow, Poland.

February 1

Thank you very much for your recent letter. I have great pleasure to comply with your request and enclose a short review of the present status of hematology in Poland. Before giving you the review I should like to tell you what conditions we worked in during the difficult times of the German occupation.

Doubtless you know that for seven years our colleges and universities were closed. Our professors were arrested by a mean trick. They were called to the university allegedly to discuss the opening of a new academic year and were there arrested and sent to concentration camps in Sachsenhausen, Sept. 9, 1939. Many of them never returned; they died the death of martyrs. In other towns it was the same. For instance in Lwów many professors were killed. Our scientific institutes were either destroyed or their equipment was appropriated and taken to Germany; now with the help of UNRRA they have been re-equipped. Our scientists if they escaped concentration camps were deprived of the facilities necessary for work but risked their lives in carrying on secret instruction in the "Underground University." I am sure you know to what dangers and persecution non-Aryan persons were exposed. A handful of them was saved in this country, thanks to friends who risked their lives to help them. It is not surprising that under the circumstances any development was virtually impossible, nevertheless the scientific achievements deserve mention. I present these achievements in outline, giving in turn the names of our four Universities and other centres.

The leading Polish hematologist is Professor Tadeusz Tempka. He was one of the first in the world to introduce Arinkin's method of sternal puncture biopsy. The results were published and are well known and often quoted in hematological literature. His hematological investigations are concerned primarily with Addison-Biermerian anemia. He showed that this disease is a primary bone marrow pathology; he called attention to the changes in the bone marrow and especially in the myeloid cells. He found and described the so-called "Riesensplabkernige." Then he found the existence of the Gaucher factor in normal human saliva. Besides the Addison-Biermer's disease he distinguished the primary "acastloses," the reverse of "asideroses." His biophysical investigations concern lymphadenography, splenograms. Lately he has been working on pulmograms, and the biophysical investigations of erythrocytes. During the German occupation he was deprived, as were other professors, of the possibility of continuing his work on clinical work and wrote a large *Handbook of Hematology* which is now being printed.

He and Doctor Kubiczek investigated the characteristics of the normal and pathological lymphadenography. Among a number of works that of Kubiczek on the normal and pathological lymphadenography is of great importance, as also are casuistic reports of biophysical diagnosis of diseases such as kala-azar which appeared here during the repatriation of the population from the East, the syndrome of Parvovirus, and during life by the help of the pulmogram, and many others presented at the meeting of the Medical Association in Cracow, 1946.

Dr. Japa Josef after his return from England is continuing his investigations of malaria in blood and other blood disorders. He introduced a new aceto-carmin staining technique. He also made contributions to the development of megakaryocytes and he put forward a new theory explaining the mechanism of hematopoiesis in pernicious anemia. Under the direction of Professor Tempka, J. Aleksander, M. Spis, and J. Koszciuszewski are working on the elaboration of the method of investigating the structure of reticular tissue by means of the intrasternal injection of India ink solutions and other methods.

nated substances. As a result of these experiments they observed that the phagocytosis by histocytes and granulocytes of the ink was weaker during infectious diseases.

In his latest studies, J. Aleksandrowicz has written on "Myelosis Erythroblastica" and on a case of retiothelio-sarcoma diagnosed during life by means of lymphadenograms. His latest work is a monograph, "Disorders of the Blood-forming Organs in the Light of Bioptical Investigation of Bone Marrow, Spleen and Lymphodes," in which he puts forward among others the proposal of unification of hematological terminology and classification of blood disorders, and gives his view on the histiogenesis of certain blood cells.

In the Biological Institute, Professor Skowron and his collaborators are investigating the influence of thiouracil on the blood forming centres of rabbits.

In the Dermatology Clinic, Prof. Walter Franciszek and Lejman Kazimierz worked out a method for the bioptical investigation of skin diseases with the aid of hemodermogram.

Prof. Kowalczykowa Janina, the head of the Anatomic-Pathologic Institute, elaborated the problem of mycosis fungoides. The basis of this disease is the overgrowth of the active mesenchyma which gives this disease a character bordering between inflammation and neoplastic processes. A similar position is occupied by Kaposi's sarcoma. With Dr. J. Cetnarowicz, she described an atypical reaction of the R-E system under the influence of carbon monoxide intoxication. With B. Skarzynski, J. Aleksandrowicz, and J. Japa she is carrying on control investigations on the characteristic leukemic reactions following the injections into animals of extracts of human organs affected by leukemia.

In the Pathology Institute, T. Rymar investigated the changes in the morphology of platelets in human and artificial animal anemias.

In my next letter I shall send you a review of Polish hematology at our other universities.

Yours sincerely,

D. Julian Alexandrowicz, M.D.

An organizational meeting of the proposed national society for the study of blood, sponsored by Dr. Alexander S. Wiener, New York City, will be held at the Hotel Claridge, Atlantic City, Sunday, June 8, at 1:30 p.m.

# ABSTRACTS

JOSEPH F. ROSS, *Editor*  
ABSTRACTERS

CHARLES P. EMERSON, Boston  
ROBERT S. EVANS, San Francisco  
OLIVER P. JONES, Buffalo

SOLOMON ESTREN, New York  
CLEMENT A. FINCH, Boston  
LAWRENCE E. YOUNG, Rochester

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## HEMORRHAGIC DISEASE

A PHARMACOLOGICAL AND RADIOLOGICAL STUDY OF HEMOPHILIA. *M. Ostro and D. I. Miller* S 39: 860-867, 1946.

The authors studied the effect of irradiation on the blood of animals and a hemophilic patient *in vitro* and *in vivo*, and came to the conclusion that the general effect of x-rays is to reduce the time of the blood. The determination of coagulation time was made by the use of venous blood in glass test tubes. Certain parallel studies—prothrombin time, platelet count, calcium and fibrinogen—showed that the change in the coagulation time was not accompanied by changes in these factors and could not be explained on that basis.

The effects were similar whether irradiation was applied to the blood after it had been placed in a test tube, or to the individual before the blood was drawn. Splenic irradiation in 2 cases was of especial interest: the application of 189 r over the spleen of the first patient resulted in a decrease in the coagulation time from 145 minutes before treatment to 15 minutes five days after treatment and 13 minutes six days later. In the other patient, in whom the initial coagulation time was 135 minutes, after irradiation was 90 minutes.

It is obvious that certain variations in coagulation time occur on different determinations, and the coagulation time of a given hemophilic may vary markedly from test to test. It is of great interest, however, that in virtually all instances here reported, both in animals and in human beings, the time change was in the direction of reduction after irradiation. In the most marked case, it was of the order of 90 per cent (145 to 15 minutes). Further and more elaborate experimental lines of this report are certainly necessary, including control studies of the coagulation time of normal patients who are receiving irradiation. If these results are confirmed, irradiation may be used as a weapon in certain acute hemorrhagic emergencies, notably those of hemophilia. The possibility of an alteration in some blood plasma factor during x-irradiation would be worthy of investigation.

## THE SPLEEN AND SPLENIC DISEASE

DISORDERS OF THE SPLEEN WITH SPECIAL REFERENCE TO THOSE AMENABLE TO SURGICAL TREATMENT. *Elliot*: Bull. New York Acad. Med. 22: 415-427, 1946.

Elliot presents an up-to-date report on the experience of the Spleen Clinic of the Presbyterian Hospital in New York which has for some years been followed with interest by many hematologists.

Best results from splenectomy have been obtained in spherocytic hemolytic anemia. Of 100 cases in this group, anemia recurred in only 1 case and was attributed to the presence of accessory spleens. The author bases prognosis from splenectomy in hemolytic anemia largely on the presence or absence of accessory spleens.

spherocytes. The fact that spherocytes may be found, however, in apparently acquired hemolytic anemia as well as in the congenital disease is not stressed. It is of interest in this connection that Boorman, Dodd, and Loutit (*Lancet* 1: 812-814, 1946) have found that red cells from patients with certain acquired hemolytic anemias are agglutinated by anti-human-serum rabbit serum whereas cells from patients with congenital hemolytic icterus are not so agglutinated. It is the reviewer's belief that the rabbit serum test may prove to be a valuable adjunct to observations on spherocytosis in estimating prognosis from splenectomy.

The results of splenectomy in thrombocytopenic purpura in patients followed in the Spleen Clinic were less satisfactory than in hemolytic icterus, but an arrest of the disease was obtained in approximately 85 per cent of 58 cases. Results from splenectomy were uniformly poor in atypical purpura subsequently found to be secondary to previously unrecognized underlying disease.

In congestive splenomegaly, best results from splenectomy were obtained in cases in which the obstructive factor could not be determined and in the group caused by schistosomiasis. In cases due to Laennec's cirrhosis or extrahepatic obstructive factors, more than one-half of the patients in the series have died, but in those with cirrhosis life expectancy was increased from 2 to 5 years by splenectomy.

Elliott stresses the inadvisability of delaying surgery where indicated once a definite diagnosis has been made.

L. E. Y.

THE SURGICAL SIGNIFICANCE OF THE ACCESSORY SPLEEN. *G. M. Curtis and D. Movitz*. *Ann. Surg.* 123: 276-289, 1946.

In the authors' series of 178 consecutive patients operated upon for various splenic diseases, 56, or 31 per cent, were found to have accessory spleens. Of the patients with accessories, varying from 1 to 8 in number, 85 per cent had them in a single location. In the 8 cases of double location, the hilus was always one of the sites. The various locations of accessories (hilus, pedicle, omentum, left gonad, etc.) are discussed in relation to the embryology of the spleen.

Curtis and Movitz state that the decreasing incidence of accessories with advancing age is a reflection of gradual involution and atrophy which normally occur. The onset of pathologic processes in splenic tissue, however, apparently causes the accessory to remain, and thus occurs the increased frequency of accessories in patients with certain splenic diseases.

The 2 recurrences of disease in the authors' series of splenectomies are reported in detail. One patient with congenital hemolytic icterus responded well to removal of the major spleen; hemolytic anemia recurred 4 years later and was completely relieved by removal of 2 accessory spleens. One patient with primary thrombocytopenic purpura had a recurrence 2 years after removal of the major spleen; death occurred from severe bleeding and accessory spleens were found at necropsy.

From the literature, the authors cite 2 additional cases of recurrence of primary thrombocytopenic purpura due to accessory spleens, and 2 cases of torsion of the pedicle of accessory spleens.

L. E. Y.

SPLENECTOMY; REMOVAL OF A SPLEEN WEIGHING 5,450 GRAMS. *J. L. Madden and C. H. Appleberry*. *Ann. Surg.* 124: 524-531, 1946.

The removal of a spleen, enormously enlarged as a result of chronic malarial infection, is described and comments are made on the surgical management of large spleens which cause symptoms by pressure upon neighboring structures.

A total of 8,000 cc. of stored, citrated whole blood transfused preoperatively to this patient, a 27 year old Filipino woman, over a period of 16 days raised the red blood cell count from 1,630,000 to only 2,330,000. During the remaining 5 days prior to operation, transfusion of 1,000 cc. of whole blood raised the red cell count to 3,930,000. The poor initial response to transfusion is attributed by the authors to two possible factors. (1) destructive hemolytic action of the spleen, and (2) the use of stored blood (age and conditions of storage not given). The authors conclude that the response of the patient is the most reliable guide in estimating the amount of blood to be transfused prior to splenectomy. They advocate administration of fresh blood during and after operation rather than massive preoperative transfusions. The customary injection of epinephrine for the purpose of contracting the spleen and autotransfusing the patient was omitted in this case because of the danger of transferring (partially?) hemolyzed erythrocytes into the systemic circulation.

It seems possible that the peculiar response to transfusion in this patient might have to sequestration of red cells in the liver which enlarged greatly during the initial period of administration. No mention of icterus is made. In the reviewer's experience, some patients with hemolytic anemia and splenomegaly have responded well to preoperative transfusions; cells have survived in a normal manner.

**SPLENECTOMY IN THE TREATMENT OF THE RHEUMATOID TYPE OF ARTHRITIS.** F. BACH, *Proc. Roy. Soc. Med.*, 39: 10-11, 1946.

Removal of the enlarged spleen in cases of Felty's syndrome (rheumatoid arthritis with neutropenia) has often been done in an attempt to return the hematologic values to normal. In these cases it is generally agreed that the operation has no effect on the underlying arthritis.

In 1940 Bach reported the removal of a *nonenlarged* spleen in 3 cases of active rheumatoid arthritis and claimed slow and sustained improvement in 2 cases. In the present article Bach reports on a patient with active advanced rheumatoid arthritis, a normal blood count, and a normal spleen. Splenectomy was followed by immediate general improvement with a marked reduction in joint pain and muscle spasm.

The rationale of the procedure, the author admits, is obscure. He has performed the operation in the selected cases and believes the results encouraging (in some cases the follow-up has been 1 year). The author notes that rheumatoid arthritis is known to improve during starvation, pruritus, type of jaundice, all of which are associated with increase in the serum cholesterol, and that splenectomy is also followed in certain cases by increase in the serum cholesterol. He also notes the causal relationship between cholesterol and various antiarthritic drugs.

The argument remains unconvincing. Check of the author's 1940 references to the literature shows that splenectomy was done only in cases in which the spleen was clinically palpable, and that joint improvement was regularly mentioned immediately after operation, 3 of the cases within 35 days to 18 months after splenectomy. The cause for the splenomegaly in these cases remains obscure. The bulk of evidence is that removal of the hyperactive spleen—although it may improve anemia, neutropenia, thrombocytopenia, and their attendant complaints—has no effect on the arthritis. The removal of the nonhyperactive spleen does not seem justified.

**HYPOPLASTIC ANEMIA: IMPROVEMENT FOLLOWING SPLENECTOMY.** L. LEVY, II, and P. PETERSON, *J. Clin. Invest.*, 39: 981-985, 1946.

The title of this paper is a misnomer, inasmuch as the case reported is one of idiopathic thrombocytopenia (panhematopenia). The patient was a 64 year old man who had previously been healthy and who complained of bleeding from the rectum and gums, and into the skin, for three months. Physical examination revealed petechiae and ecchymoses, and splenomegaly. The blood picture showed severe anemia, neutropenia, and thrombocytopenia. The bone marrow was not hypoplastic, but rather showed normal development of erythrocytes and granulocytes. The spleen weighed 410 grams at operation and was found to contain only congestion on pathologic examination. Splenectomy was followed by elimination of the bleeding and a return of the blood counts to essentially normal values.

Three points are of especial interest. (1) Injection of adrenalin gave no significant change in the blood count. This is in contrast to the experience of Doan with the adrenalin test, but in contrast to the experience of others. (2) The bone marrow showed large numbers of granulocytes despite their marked reduction in the peripheral blood. (3) Supravital studies of the excised spleen showed no erythrocytopenia. The authors point out, however, that of the 10 cases of splenic neutropenia in the literature, 7 showed neutrophagocytosis in the spleen.

This case is another instance of "hypersplenism"—splenic neutropenia, anemia, or thrombocytopenia. In this instance, all three bone marrow elements were affected, resulting in pancytopenia. The term "hypoplastic" is to be deplored for these cases, inasmuch as the marrow shows no evidence of hyperplasia of the involved elements.

BANTI'S SYNDROME FOLLOWING PROLONGED INFECTIOUS HEPATITIS. R. L. Fisher and M. Zukerman. *Am. J. Digest. Dis.* 13: 361-366, 1946.

Banti's disease as originally described is considered to be an entity of unknown etiology characterized by splenomegaly, anemia, leukopenia, gastrointestinal hemorrhages, and, later, cirrhosis of the liver. The cause of the disorder has been obscure; and, in recent years, the existence of such a specific disease has been doubted. The term "Banti" has rather been applied to virtually any disorder in which splenomegaly has been associated with otherwise unexplained anemia and leukopenia.

The authors of the present article describe what they consider to be a new cause of "Banti's syndrome." They report 2 cases of acute infectious hepatitis in which, subsequently, persistent splenomegaly, anemia, and, in 1 case, pancytopenia were discovered. In 1 case splenectomy was done with moderate improvement. The authors conclude that a virus may be responsible for certain instances of Banti's syndrome, inasmuch as infectious hepatitis is considered to be of virus etiology, and recommend splenectomy as soon as splenomegaly is found following hepatitis.

Anemia, leukopenia, and pancytopenia have been described in a variety of unrelated conditions in which the spleen becomes enlarged. In portal cirrhosis this enlargement is interpreted as due to portal congestion; and the same explanation probably holds for acute hepatitis, the postulate of a virus etiology probably being unjustified. Routine splenectomy in such cases does not seem warranted, but in selected cases it may be counted upon to eliminate anemia, neutropenia, and pancytopenia.

S. E.

SPONTANEOUS RUPTURE OF THE SPLEEN IN SARCOIDOSIS. I. James and A. J. Wilson. *Brit. J. Surg.* 33: 280-282, 1946.

Rupture of the spleen has been reported in recent years with increasing frequency. The underlying cause is usually malaria, sometimes infectious mononucleosis; and rarely no cause can be found. In the present report, severe epigastric pain and vomiting occurred in a 49 year old man, and the presence of a tender and rigid abdomen led to a diagnosis of peritonitis of obscure origin. At operation a spleen was found which seemed normal except for multiple hemorrhagic areas and one large rent through the upper pole; bleeding from this rent had resulted in a moderate amount of fluid and clotted blood in the peritoneal cavity. Pathologically, the spleen disclosed many pulp and subcapsular hemorrhages; but the unexpected finding was the presence of the typical lesions of Boeck's sarcoid. Subsequent investigation of the patient failed to reveal other evidence of sarcoid in the eyes, skin, bones, lungs, or lymph nodes.

The presence of signs of obscure intraperitoneal hemorrhage should suggest the possibility of splenic rupture, even if the spleen was not previously known to be diseased. Sarcoidosis must be added to the list of causes of splenopathy which may be followed by spontaneous rupture.

S. E.

## SEROLOGY

FALSE POSITIVE SEROLOGIC TESTS FOR SYPHILIS FOLLOWING BLOOD DONATION. R. D. Barnard, C. R. Rein, and C. A. Doan. *Am. J. Syph. Gonorr. & Ven. Dis.* 30: 255-263, 1946.

The occurrence of transiently positive serologic tests for syphilis in several repeat blood donors with no other evidence of syphilis led the authors to investigate the incidence of false positive serologic tests at the Red Cross Center in Columbus, Ohio. It was confirmed that a certain number of donors, initially seronegative, became seropositive after giving from one to four donations. An incidence of 0.4 per cent of such serologic reversal occurred among some 28,000 repeat donors. Reversal was rarely seen after the fifth or subsequent donations. The positive serology occurred from 10 days to 3 weeks after a particular blood donation, and disappeared in from 1 week to 4½ months later.

The authors point out that, although the incidence of this aberration is small, the actual number of cases must have been large because of the millions of blood donations given during the war years. A knowledge of the occurrence of the phenomenon will prevent unnecessary antisyphilitic treatment (which was actually given in several cases described) and eliminate premature and incorrect diagnoses of lues. It is of great theoretical interest that venesection may so alter the plasma as to result in false serologic reactions. The nature of this alteration is not as yet clear.

S. E.

# BOOK REVIEWS

*Diseases of Blood Forming Organs in the Light of Biopsies of Bone Marrow, Spleen, and Lymph Nodes.* JULIAN ALEKSANDROWICZ. D. E. Friedlein, Krakow, Poland (1946). Pp. 265, with 57 illustrations, 12 in color, 45 microphotograms.

In the first chapter of this text, the technic of biopsies of the sternum, spleen, and lymph nodes is described. The anatomy and physiology of the blood-forming centers is discussed in the second chapter; the role of the nervous system, the endocrines and other organs in the third chapter. The fourth chapter deals with the preparation and evaluation of myelo-, spleno-, and lymphadenograms. Chapters 5 to 11, an original classification and a detailed description of the diseases of the blood-forming organs is given.

It is astonishing that under difficult circumstances the author has been able to gather the material for his book. The publisher is also to be congratulated on the good printing and excellent illustrations.

*La Cultura in Vitro del Midollo Osseo.* By ANITA FIESCHI AND GIOVANNI ASTALDI. Tipografia dell'Editore, Pavia, 1946. Pp. 309.

This book offers a rather complete review of the subject with close to 350 bibliographic references in the Italian, English, German, French, and Russian languages. The technic for the preparation and study of supravital cultures is given in great detail and with a practical approach. Almost half of the work is devoted to the review of the literature, the technical aspects of the problem, and the results of work in supravital preparations. Of the pathologic conditions, only pernicious anemia, megaloblastic and lymphatic leukemia, and Cooley's anemia are studied and here the progressive changes of the marrow elements as seen in *in vitro* cultures are given in great detail.

As to pernicious anemia, the "problem of the megaloblast" is "solved" with the contention that megaloblasts arise directly from "histio-endothelial elements" and not from a red stem cell common to both megaloblasts and normoblasts. The shift from megaloblastic to normoblastic marrow following liver therapy is interpreted as resulting from transformation of megaloblasts into normoblasts.

Bone marrow culture from cases of chronic myelogenous and lymphatic leukemia did not reveal morphologic or developmental abnormalities which could be used as differentiating normal from leukemic processes. In acute leukemia, hemopoiesis was more deeply altered; the largest number of "myeloid cytoblasts" showed no evolution toward either myeloid maturation or fibroblast formation.

The authors believe that their observations of the marrow from cases of Cooley's anemia point to a deficiency in red blood cell maturation based on a primary marrow lesion. This abnormality appears to result from nuclear rather than cytoplasmic deficiency.

The work would be difficult to read for anyone not familiar with the nomenclature of the Italian hematologic school. The style tends to be heavy and prolix. In so far as the monograph contains much scattered material it represents a valuable contribution to the literature of hematology. The micrographs and colored plates are numerous, the latter are beautifully lithographed and serve well from the standpoint of illustrating the text material.

*Disorders of the Blood.* By SIR LIONEL E. H. WHITBY AND C. J. C. BRITTON. The Blackiston Company, Philadelphia, 5th edition. Pp. 665. \$10.00.

This fifth edition is an expanded and thoroughly revised version of one of the standard reference texts in hematology. Comparison with the earlier work reveals much new material in the chapters on the hemolytic anemias, hematologic immunology, the anemias of infancy and childhood, blood transfusion, and technic. The text has been increased by sixty-two pages and four new chapters on the diseases of bone marrow have been added.

The systematic subdivision of the material into well-defined cubbyholes and the change of punctuation

which marked the earlier editions are well maintained. Of especial note are the meaty and concise summaries at the end of each chapter, the careful subject and author indexes, and the critically chosen references, which are mainly to key papers. The format is pleasing and the colored plates are good although one could wish for a few more colored illustrations.

Although the aim of the authors is to integrate disorders of the blood with the general field of internal medicine, the increasing volume of knowledge in this specialized field has necessarily limited the discussion to hematology and closely allied subjects.

The discussions on therapy might well be expanded and the frequent use of proprietary British pharmaceuticals as examples curtailed. A more critical analysis of some of the newly proposed procedures in immunohematology, as, for example, the transfusion of 50 cc. of blood as an *in vivo* test of compatibility, might have been expected.

On the whole, the relative completeness of the book, its clarity of style, and the exposition of most of the recent advances mark it as a very welcome addition to the library of students, practitioners, and internists interested in keeping abreast of a rapidly expanding field, and as a valuable ready reference textbook. The chapter on methods, particularly that part dealing with the Price-Jones curves, is particularly good.

*Practical Malariology.* By PAUL F. RUSSELL, M.D., M.P.H., LUTHER S. WEST, Ph.D., AND REGINALD D. MANWELL, Sc.D. Prepared under the auspices of the Division of Medical Sciences of the National Research Council. W. B. Saunders Co., Philadelphia and London, 1946. Pp. 684. Price \$8.00.

This is a manual originally designed for the use of the American armed forces. It was undertaken in the closing months of the war and was completed in the postwar period. Although originally intended as a military manual, it has been rewritten primarily from the standpoint of civilian needs.

The writing is done by a well known malarialogist, Colonel Russell, who was aided by an entomologist, Dr. West, and a protozoologist, Dr. Manwell. A happier combination could hardly have been conceived.

The work is distinguished by its eminent practicality, and by the enormous amount of very readable information which is compressed into a relatively small space. There are certainly no wasted words, and it is refreshing to see things put down in clear and lucid fashion. The diagrams, charts, and figures are well conceived. There are some excellent colored plates of parasites. The book is divided into six sections as follows: The Parasite, The Mosquito, The Man, The Community, Prophylaxis and Control, and Therapeutic Malaria. The very practical chapters on "Field Technic" and "Laboratory Technic" with exact directions for collection and study of specimens are, in their particular fields, classics. There is a very interesting chapter on "The Spleen Index" in which palpation and measurement of the spleen are thoroughly discussed. Hackett's suggestions for classifying enlargement of the spleen from 0 to 5 are followed.

In the section on prophylaxis and control, there is a very complete chapter on larvicides with particular reference to D.D.T. A thorough review of the chemical nature, methods of using this substance, toxic effects, etc., is presented. A seventy page appendix deals with the world anophelinae.

It is a pleasure to recommend this work to anyone interested in the study and control of malaria, which by many is considered to be the world's most important disease.



# BLOOD

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### DEVELOPMENT OF INCLUSION BODIES CONTAINING RIBOSE NUCLEIC ACID IN MYELOMA CELLS AFTER INJECTIONS OF STILBAMIDINE. DETERMINATION OF STILBAMIDINE IN MYELOMA TISSUE

By I. SNAPPER, M.D., A. E. MIRSKY, PH.D., H. RIS, PH.D.,  
B. SCHNEID, M.D., AND M. ROSENTHAL, M.D.

IN CASES of multiple myeloma, treatment with stilbamidine often results in special morphologic alterations in the bone marrow.<sup>1</sup> Whereas myeloma cells of untreated patients never contain basophilic granules (fig. 1), after injection of stilbamidine,\* basophilic inclusion bodies appear in the cytoplasm of the myeloma cells of many patients. In none of the other cellular components of the marrow have such inclusions been found. These inclusion bodies are stained deep blue with Giemsa or Wright stain and bright red with Unna's phenol methyl green pyronine. In supravital stained preparations exposed to Janus green and neutral red the myeloma cells are seen to contain large globules stained by neutral red.

The staining properties of these inclusion bodies—deep blue with Giemsa, deep red with pyronine methyl green—allow certain deductions about their chemical composition.

It has been known for many decades that the nucleoproteins of cells display pronounced basophilia. Nucleoproteins consist of protein conjugated with nucleic acid; the basophilia of nucleoproteins is due to the nucleic acid content. This knowledge has been expanded in recent years and it is now realized that the basophilia of many cellular inclusions depends upon their nucleoprotein content.<sup>2-4</sup>

Recently, chemical and physical methods have been made available by which nucleoproteins can be studied in the intact cell. These methods also permit differentiation between the desoxyribose nucleic acid present in the nucleus and the ribose nucleic acid which is present in the nucleoproteins of cytoplasm and nucleolus.

1. With Unna's phenol methyl green pyronine solution, the ribose nucleic acid of cytoplasm and of the nucleolus stains red, the desoxyribose nucleic acid of the nucleus blue green.

2. The enzyme ribonuclease which was used by Dubos<sup>5</sup> and Brachet<sup>6</sup> and later crystallized by Kunitz<sup>7</sup> depolymerizes ribose nucleic acid but does not attack desoxyribose nucleic acid. After cells have been exposed to the action of this enzyme, structures containing ribose nucleic acid no longer stain.

From the Second Medical Service of the Mount Sinai Hospital and the Laboratories of the Rockefeller Institute for Medical Research.

\* The stilbamidine used in these experiments was donated by Merck & Co., Inc., Rahway, N. J., through the courtesy of Dr. D. F. Robertson.

3. Lanthanum salts precipitate nucleoproteins. This fact has been used by Caspersson, Hammarsten, and Hammarsten<sup>8</sup> for the analysis of nucleic acids. Caspersson<sup>9</sup> used lanthanum salts to fix the desoxyribose nucleic acid of chromosomes, and recently Opie<sup>10</sup> has applied this technic to study the ribose nucleic acid of cytoplasm and of basophilic cytoplasmic inclusions. After tissue sections have been subjected to the action of lanthanum salts the ribose nucleic acid cannot be removed by ribonuclease, as proved by staining with Giemsa or with methyl green pyronine solution.

4. Nucleoproteins show a specific ultraviolet absorption. The use of the ultraviolet microscope has made it possible to photograph the nucleoproteins in the



FIG. 1. MYELOMA CELL OF UNTREATED PATIENT. BASOPHILIC, SOMEWHAT VACUOLATED PROTOPLASM. GIEMSA STAIN

and unstained cells.<sup>11</sup> As the ultraviolet absorption of nucleoprotein depends on the purine and pyrimidine content of nucleic acid, this method does not differentiate between ribose nucleic acid and desoxyribose nucleic acid, as both substances have the same ultraviolet absorption.

#### METHODS AND RESULTS

Bone marrow smears were fixed by exposure to heat at 160 degrees for 20 sec. Thereafter the smears were dipped in methyl alcohol for 1 minute. Different smears were treated in the following way.

a. After fixation, smears were immersed for 15 and for 30 minutes in distilled water at 28 degrees C.

b. After fixation, smears were immersed for 15 and for 30 minutes in a solution of ribonuclease\* 0.04 per cent at a temperature of 28 degrees C.

\* We are greatly indebted to Dr. M. Kunitz, who has kindly put a generous quantity of ribonuclease at our disposal.



FIGS. 2A AND 2B. MYELOMA CELLS OF PATIENT TREATED WITH STILBAMIDINE FOR 6 WEEKS  
Fixation: 20 seconds at 160°, followed by 1 minute in methyl alcohol. Giemsa stain. Large basophilic inclusion bodies.

c. After fixation, smears were exposed to a fifth molar lanthanum acetate solution for 2 hours. The smears were thoroughly washed and then exposed for 15 and for 30 minutes to ribonuclease 0.04 per cent at a temperature of 28 degrees C.

All smears were then stained with Giemsa solution. Another smear was used as a control and was stained with Giemsa solution immediately after fixation.

The control smears stained after fixation contained many myeloma cells with basophilic cytoplasm and deeply blue-stained inclusion bodies (figs. 2A and 2B). After immersion in double distilled water at 28 degrees for 30 minutes the myeloma cells were scarcely changed, although occasionally the basophilia of the cytoplasm was slightly diminished. The inclusion bodies, however, had not been touched and stained deep blue (fig. 3).

After enzymatic digestion in ribonuclease solution the cytoplasm of practically all the myeloma cells had disappeared. The inclusion bodies also had vanished.



FIG. 3. BONE MARROW SMEAR OF MYELOMA PATIENT TREATED WITH STILBAMIDINE FOR 6 WEEKS. Fixation 20 seconds at 160° followed by 1 minute in methyl alcohol. Immersed for 30 minutes in double distilled water at 28°. Giemsa stain.

Basophilic inclusion bodies well preserved.

Here and there a myeloma cell still showed the presence of faintly stained granules (fig. 4).

For completeness it may be added that the nucleoli of the myeloma cells were also digested by ribonuclease. As mentioned above, nucleoli in contrast to the rest of the nucleus contain considerable amounts of ribose nucleic acid.

When immersion in lanthanum acetate solution was followed by ribonuclease digestion, the cytoplasm of the myeloma cells could still be stained dark blue with Giemsa solution; the same held true for the inclusion bodies (fig. 5).

It is possible to obtain comparable results if, instead of Giemsa, Unna's phenol pyronine methyl green solution is used. With the latter stain cytoplasm, inclusion bodies, and nucleoli of the myeloma cells take a deep red color. However, Unna's stain if applied to bone marrow smears is erratic and stains the thicker part of the

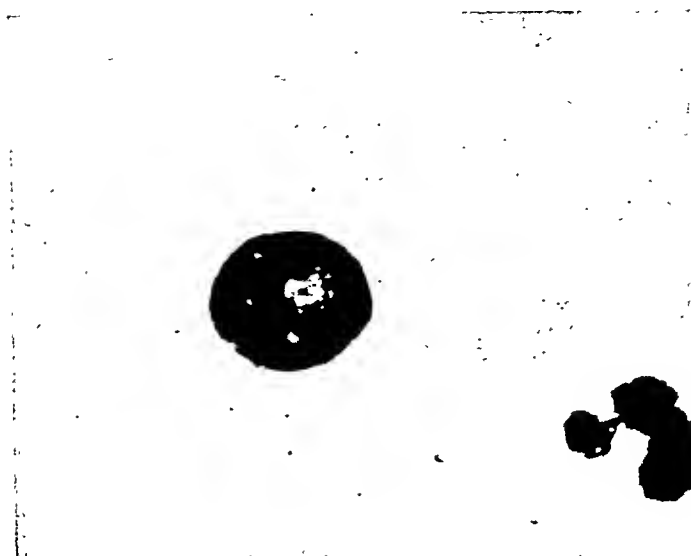


FIG. 4. BONE MARROW SMEAR OF PATIENT WITH MULTIPLE MYELOMA TREATED WITH STILBAMIDINE

Fixation: 20 seconds at 160°, then 1 minute in methyl alcohol.

Smear immersed in ribonuclease solution (Kunitz) 0.04 per cent for 30 minutes.

Giemsa stain. Basophilia of cytoplasm and inclusion bodies completely disappeared.

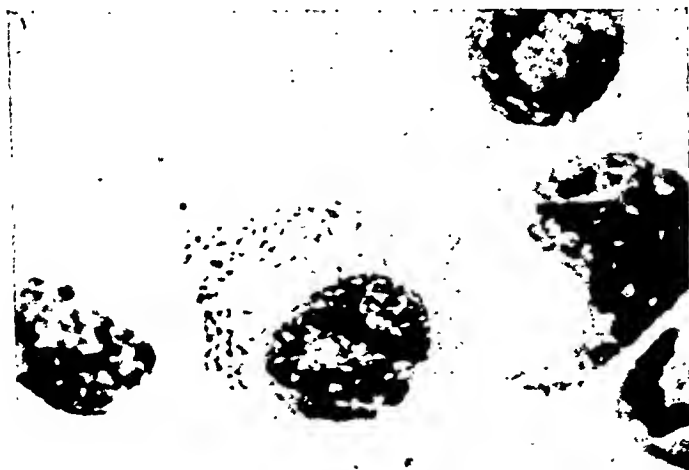


FIG. 5. BONE MARROW SMEAR OF PATIENT WITH MULTIPLE MYELOMA TREATED WITH STILBAMIDINE

Fixation: 20 seconds at 160°, then 1 minute in methyl alcohol.

Smear immersed for 2 hours in  $\frac{1}{2}$  molar watery solution of lanthanum acetate. Then for 30 minutes in ribonuclease solution (Kunitz) 0.04 per cent at 28°.

Giemsa stain. Basophilia of cytoplasm and inclusion bodies well preserved.

smears much more intensely than the thinner parts. The pyronine stain may, therefore, give rise to erroneous conclusions if used to compare the ribose nucleic acid content of myeloma cells before and after digestion with ribonuclease.

The ribonuclease method as used for the demonstration of ribose nucleic acid in the myeloma cells of bone marrow smears differs slightly from the methods published recently. Up to the present time ribonuclease has been used mainly to demonstrate the presence of ribose nucleic acid, either in solutions or in microscopic specimens. The latter had been fixed in Zenker's or Helly's solution. The ribonuclease solution was usually allowed to act for 1 or more hours at temperatures ranging between 37 degrees and 70 degrees; Bracher<sup>6</sup> used 1 hour at 70 degrees, Davidson and Waymouth 5 hours at 37 degrees, Opie<sup>10</sup> 2 hours at 56 degrees, Deane<sup>12</sup> 3 hours at 60 degrees. The bone marrow smears we studied consist of a unicellular layer, much thinner than the microscopic specimens which were used by most authors. It is evident that under these circumstances the action of ribonuclease on the thin bone marrow smears had to be limited to shorter periods and to lower temperatures than are customarily used in the study of microscopic specimens. This explains why our experiments on the influence of ribonuclease on bone marrow smears were performed at a temperature of 28 degrees and why the time of action was limited to 30 minutes.

The choice of a method of fixation is not of great importance. As mentioned, we used heat and methyl alcohol, but comparable results can be obtained after fixation of the bone marrow smears with Zenker's fluid. Since the staining of fine cytoplasmic structures in these smears is occasionally unsatisfactory after the use of Zenker's fluid, we record only the results obtained with smears fixed by heat and methyl alcohol.

We had occasion, however, to examine microscopic sections of the bone marrow of one myeloma patient who had been treated with stilbamidine and who died of thrombopenic purpura. Phenol methyl green pyronine solution stained the cytoplasm of the myeloma cells a vivid red, and in addition revealed the presence of groups of deeply red stained granules in and around the myeloma cells. After preliminary immersion of the specimens in 0.02 per cent ribonuclease solution for 2 hours at 40 degrees neither the cytoplasm of the myeloma cells nor the granules stained with pyronine.\*

We found it advisable to dissolve the ribonuclease in twice-distilled water and not in a buffer as has been done by most of the other workers. In our experience the ribose nucleic acid of the myeloma cells and of the inclusion bodies present in bone marrow smears dissolves much more easily in a buffer of pH 6.75 to 7.4 than in twice-distilled water.

It is evident that the removal of the basophilic clumps of the myeloma cells from bone marrow smears and bone marrow sections under the influence of ribonuclease makes it highly probable that these inclusion bodies contain ribose nucleic acid.

Ultraviolet microscopy was used to confirm the results obtained with ribo-

\* These microscopic specimens of the bone marrow were prepared and stained by Dr. P. Klemperer of the Mount Sinai Hospital. We gladly take this occasion to thank Dr. Klemperer for his continuous interest and support.

nuclease because ribose nucleic acid absorbs ultraviolet radiation of 2,600 Å. Bone marrow smears were prepared on quartz slides and were fixed in methyl alcohol. Photomicrographs of these smears were made with a quartz microscope using a light source which emitted ultraviolet light of 2,600 Å. In these photomicrographs the myeloma cells contained large black granules (fig. 6). The fact that in the inclusion bodies of the myeloma cells a substance with a specific ultraviolet absorption of 2,600 Å. can be revealed confirms the results obtained with ribonuclease. This finding, in conjunction with the results of the other methods mentioned above, provides strong evidence for the presence of ribose nucleic acid in the inclusion bodies of the myeloma cells. This does not necessarily mean that these inclusion bodies consist exclusively of ribose nucleic acid or of ribose nucleoprotein.

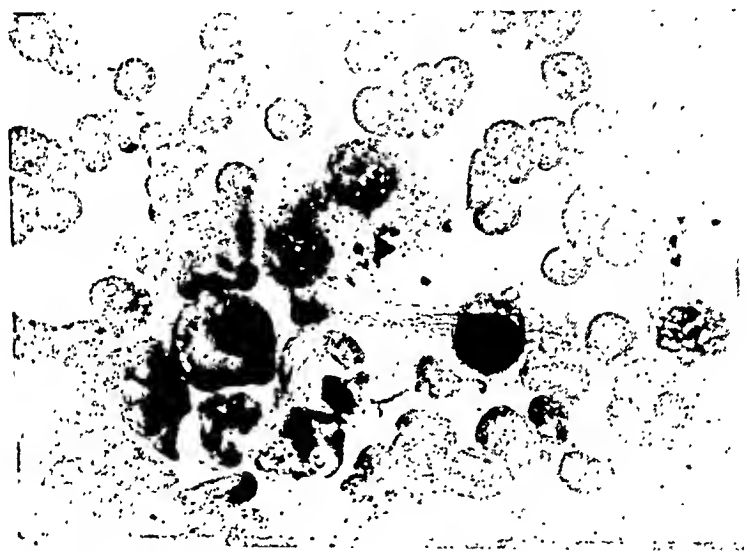


FIG. 6. PHOTOGRAPHS TAKEN WITH QUARTZ MICROSCOPE OF BONE MARROW SMEARS OF MYELOMA PATIENT TREATED WITH STILBAMIDINE

Light source 2,600 Å. Bone marrow smeared on quartz slide. Myeloma cells showing inclusion bodies.

At the same time it has been possible to demonstrate that after treatment of myeloma patients with stilbamidine this drug is present in the myeloma tissue. The myeloma tissue was obtained at the autopsy of a patient who died after she had received a total of 5,175 milligrams of stilbamidine in the course of six weeks. The last stilbamidine injection had been administered eight days before death.

For the determination of stilbamidine in the myeloma tissue an extract of the tumor mass was prepared by emulsifying it in 10 cc. of 0.9 per cent NaCl. After centrifugation the sediment was washed twice with 10 cc. of 0.9 per cent NaCl. This washed sediment was suspended in 10 cc. of 0.9 per cent NaCl and subjected to a final slow centrifugation.\* The supernatant fluid of this fraction was analyzed.

\* We are greatly indebted to Dr. G. Schwartzman of the Mount Sinai Hospital for his willingness to prepare these extracts of the myeloma tissue under adequate bacteriologic controls.

The detailed procedure was as follows:

The myeloma tissue was mixed with 10,000 units of penicillin and kept for 1 hour at 37° C. Cultures showed the presence of gram-negative organisms which did not grow out in subcultures.

The mixture was then kept for 21 days in the deep freeze. It was thawed and the penicillin was removed. The myeloma tissue was ground in a mortar over dry ice and the emulsion suspended in 10 cc. of 0.9 per cent NaCl. This suspension was placed for 24 hours in the deep freeze, then thawed and centrifuged at 3,000 R.P.M. for 10 minutes. The supernatant fluid A was saved in the refrigerator.

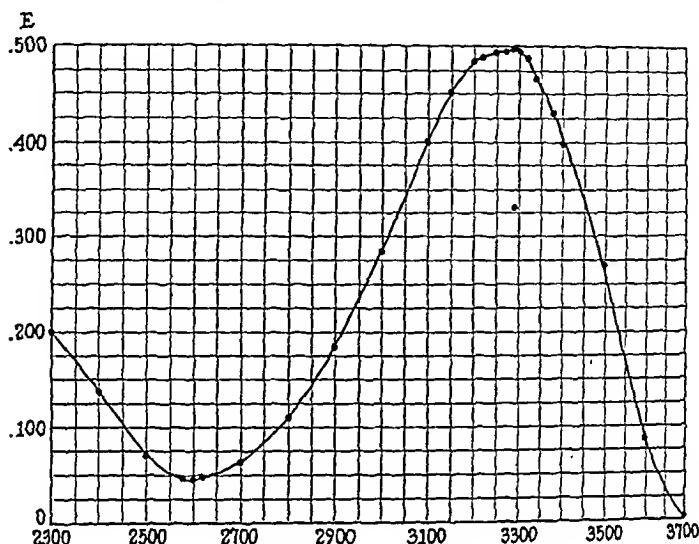


FIG. 7. ULTRAVIOLET ABSORPTION CURVE OF AN AQUEOUS SOLUTION OF STILBAMIDINE CONTAINING 0.007 MG. PER CC.

Point marked \* represents the extinction coefficient at maximal wave-length of a solution containing 0.0035 mg. per cc.

Wave-length in Å. U.

The sediment was transferred to a mortar, ground over dry ice, and suspended in 10 cc. of 0.9 per cent NaCl. To this suspension the supernatant fluid A was added and the pooled specimen was centrifuged for 10 minutes at 1,000 R.P.M. The supernatant fluid was separated and the sediment again suspended in 10 cc. of 0.9 per cent NaCl. It was kept for 1 hour in the refrigerator and then centrifuged for 10 minutes at 1,000 R.P.M.

The sediment obtained was kept for 2 months in the deep freeze and then suspended in 10 cc. of 0.9 per cent NaCl solution, centrifuged at 1,000 R.P.M. for 10 minutes, and the supernatant fluid was used for the analysis.

The presence of appreciable quantities of stilbamidine in this extract was apparent because examination with ultraviolet light showed the presence of a strong

blue fluorescence. This blue fluorescence is one of the characteristics of stilbamidine solutions and is used for the determination of this substance in biologic fluids.<sup>13</sup>

This result was checked by spectrophotometric analysis of the extract.

Goodwin<sup>14</sup> has investigated the ultraviolet absorption of stilbamidine. In his report the absorption curve shows maximum absorption at 3,250 Å., but in a table an absorption maximum of 3,090 Å. is given. We have redetermined the ultraviolet absorption of stilbamidine solutions and found the maximal absorption to be at 3,290 Å. (fig. 7). The extinction coefficient at this wave-length is 0.498 for a solu-

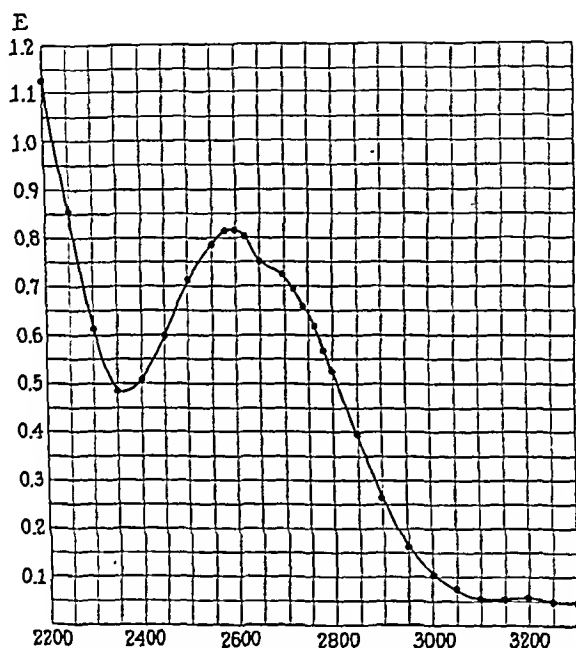


FIG. 8. ULTRAVIOLET ABSORPTION CURVE OF A SALINE EXTRACT OF MYELOMA TISSUE  
Wave-length in Å. U.

tion containing 0.007 mg. per cc. Beer's law does not hold, for a solution containing 0.0035 mg. per cc. of stilbamidine has an extinction coefficient of 0.330 (fig. 7).

Spectrophotometric analysis of the extract obtained after storage in the deep freeze shows intense absorption at 2,600 Å. due to nucleic acid and absorption at 3,200 Å. due to the presence of appreciable quantities of stilbamidine (fig. 8). For spectrophotometric determinations 1 part of this extract was diluted with 46 parts of saline. From the extinction coefficient (0.824) at 2,600 Å. the concentration of nucleic acid and nucleotides can be computed. A nucleic acid solution containing c. 1 mg. per cc. has an extinction coefficient of 2.1 at 2,600 Å. The undiluted extract accordingly contains 1.7 mg. of nucleic acid per cc. To estimate the quantity of stilbamidine present the extinction coefficient at 3,200 Å. was determined in a solution of the extract diluted with 7 parts of saline. From this value, 0.420, it is computed that the undiluted extract contains 0.037 mg. of stilbamidine per cc.

This value may be too low, for stilbamidine is unstable, but at all events the amount of stilbamidine compared to the amount of nucleic acid is very low.

#### DISCUSSION

From these results it may be concluded that not only the cytoplasm of the myeloma cells but also the basophilic inclusion bodies which develop in the myeloma cells under influence of stilbamidine contain considerable quantities of ribose nucleic acid. In addition, there is evidence that stilbamidine is deposited in the myeloma tissue.

In recent years evidence has been collected which supports the hypothesis that nucleoproteins regulate the formation of protein by cells. Cells which are engaged in rapid protein synthesis are especially rich in basophilic nucleoproteins. This can be demonstrated in cells from different organs with widely different physiologic functions such as the cells of the serozymogenic glands of the digestive system, the liver, the lymphocytes, the nerve cells, the placenta, and certain elements of the endocrine glands. Any change in the rate of the protein formation in one of these cells is accompanied by differences in the amount or distribution of the cytoplasmic basophilia.

Thorell and his associates demonstrated that the cytoplasm of myeloma cells is rich in ribose nucleic acid and also stressed the active synthesis of protein in myeloma cells.<sup>17</sup>

Since various kinds of proteins are elaborated by different cells, the corresponding nucleoproteins which regulate the formation of protein must also differ. In this connection the conception that globulins and Bence-Jones protein are manufactured within the myeloma cells becomes important. If the nucleoproteins of myeloma cells elaborate the abnormal globulins and the Bence-Jones protein, they should be different from the nucleoproteins of all other body cells.

Kopac<sup>18</sup> has suggested that stilbamidine may dissociate nucleoproteins, and he has shown that stilbamidine can in fact dissociate a protamine-ribonucleate complex. Kopac states that "in all probability the property of a compound to dissociate protamine-ribonucleate is dependent on its ability to denature the protein moiety." There is no need to suppose that "denaturation" is required to displace protamine from its combination with nucleic acid. It is true that stilbamidine combines with desoxyribose nucleic acid, forming a fibrous precipitate, as would be expected from the presence of amidine groups in stilbamidine. It has already been found that protamine which has a great affinity for nucleic acid is able to displace histone from its combination with nucleic acid in isolated nuclei and chromosomes.<sup>19</sup> In this experiment one base displaces another from a salt-like linkage. We found that when stilbamidine is added to isolated nuclei, histone is displaced. Arginine and some other amidines, on the other hand, are unable to displace histone. The ability of protamine and stilbamidine to displace histone depends upon the relative affinities of these substances for nucleic acid. As mentioned above, there is reason to believe that the nucleoproteins of the myeloma cells differ from the nucleoproteins of other body cells. Thus it seemed possible that the protein of the nucleoprotein in myeloma cells is more readily displaced by stilbam-

idine than is the protein of other nucleoproteins. Experiments like those on the displacement of histone by protamine and stilbamidine should be done on the nucleoproteins of myeloma cells and other cells. In this way, the observation that stilbamidine influences the course of multiple myeloma but has no influence upon other proliferative diseases of the blood-forming organs or neoplasms can perhaps be explained.<sup>20,21</sup>

#### SUMMARY

The cytoplasm of myeloma cells is rich in ribose nucleic acid. The basophilic inclusion bodies which develop in myeloma cells under the influence of stilbamidine contain ribose nucleic acid. The myeloma tissue of patients treated with stilbamidine contains appreciable amounts of stilbamidine.

There is reason to believe that the nucleoproteins of myeloma cells differ from the nucleoproteins of other cells. This could explain why stilbamidine reacts with the nucleoproteins of myeloma cells only, and not with the nucleoproteins of other cells.

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## THE ANEMIA OF INFECTION, VI. THE INFLUENCE OF COBALT ON THE ANEMIA ASSOCIATED WITH INFLAMMATION

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ALTHOUGH the bone marrow usually appears to be normal or even hyperplastic in cases of anemia associated with infection,<sup>1</sup> the anemia is refractory to therapy, being relieved only when the underlying infection is successfully eradicated. The anemia is accompanied by a profound metabolic disturbance as indicated by the finding of hypoferrremia, hypercupremia, and elevated erythrocyte protoporphyrin levels.<sup>2</sup> It is recognized, also, that infection is associated with a disturbance in protein metabolism; thus the serum albumin decreases, and increased excretion of urea in the urine has been reported.<sup>3</sup>

As part of a study of the pathogenesis of the anemia associated with inflammation, and in an attempt to find a means whereby the failure to form hemoglobin might be overcome, it was considered of interest to study the effect of cobalt. By the administration of cobalt, polycythemia can be produced in many laboratory animals.<sup>4,5</sup> The increase in red cells and hemoglobin has been reported as being due to an actual increase in red cell mass,<sup>6,7</sup> and an increase in reticulocytes in the blood has been observed. The mean corpuscular volume was found increased, owing mainly to greater cell thickness,<sup>7</sup> the bone marrow becomes hyperplastic,<sup>8,9</sup> and metaplasia occurs in the spleen, liver, and kidneys.<sup>9,10</sup> The administration of cobalt was found to overcome the anemia produced by the toxic action of benzol in rabbits<sup>8</sup> and that caused by protein deficiency in rats<sup>11</sup> and was even reported to relieve the physiologic and nutritional anemia of children.<sup>12</sup> Kleinberg and his associates<sup>8</sup> found that in rabbits made anemic by the injection of 0.5 to 1.0 ml. of benzene for a period of 5 weeks, the daily administration of 50 ml. of cobalt nitrate overcame the anemia despite the continued administration of benzene. The marrow of the control benzene rats was fatty and aplastic, while the marrow of the rats treated with cobalt and benzene was hyperplastic. It has been shown by Dorrance et al.<sup>10</sup> that the work performance of rats having cobalt polycythemia is increased under conditions of anoxia, thus indicating that the increased hemoglobin is useful for oxygen carriage.

### METHODS AND PROCEDURE

Rats of Sprague-Dawley strain and from the Carsworth Farms were used. Since it has been shown that sterile turpentine abscesses have the same effect in animals as chronic infection<sup>13,14</sup> this means was employed to produce inflammation. In the first experiment 24 rats were used, these being divided into 4 groups of 6 each. All rats were fed fox chow *ad libitum*. Group I were controls. Group II ("cobalt") were given 0.125 mg. cobalt (0.5 mg. cobaltous chloride,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ) intraperi-

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tonically daily. Group III ("cobalt and turpentine") were injected intramuscularly with 0.25 ml. turpentine (Rexall, U.S.P.), and this was repeated according to the clinical condition of the animals; cobaltous chloride, 0.5 mg., was given intraperitoneally each day as well. Group IV ("turpentine") were injected with turpentine as in group III but received no cobalt.

The second experiment represented a repetition of the first, only the following slight modifications being made: The diet was Purina dog chow; the first injection of turpentine was 0.25 ml. and the succeeding weekly doses were 0.125 ml. This was

TABLE 1.—*Summary of Data*

Group No.	Hemoglobin Gm./100 ml.		Vol. Pkd. RBC	Eryth- rocyte Proto- por- phyrin	Plas- ma Iron	Se- rum Cop- per	Protein				Urea & Am- monia Nitro- gen Gm./ 24 hr.	Weight, Gm.		
	Before	After	ml./ 100 ml.	mg./100 ml. packed RBC	mg./ 100 ml.	mg./ 100 ml.	Total	Al- bumin Gm./ 100 ml.	Glo- bulin Gm./ 100 ml.	A/G ratio		Before	After	Change per cent
Experiment I														
I. "Control"	13.4	16.9	50.0	33	354	231						131	190	+45
II. "Cobalt"	14.0	19.3	54.0	88	234	243						120	187	+56
III. "Cobalt & Tur- pentine"	13.5	18.7	51.0	52	214	225						110	177	+61
IV. "Turpentine"	14.1	13.8	41.0	138	161	227						118	174	+47
Experiment II														
I. "Control"	16.3	15.4	45.0	52	281	225	6.56	4.56	2.00	2.31		172	201	+15
II. "Cobalt"	16.2	19.3	56.0	82	262	282	6.32	4.14	2.18	1.90		167	193	+15
III. "Cobalt & Tur- pentine"	15.9	18.2	52.0	113	184	256	5.47	3.22	2.25	1.43		181	179	-2
IV. "Turpentine"	17.3	14.5	41.0	85	188	282	5.42	3.50	1.92	1.83		183	201	+10
Experiment III														
I. "Control"	16.1	16.9	49.3	36	260	179	5.95	3.70	2.23	1.64	0.162	161	208	+29
II. "Cobalt"	16.1	22.1	62.3	62	209	225	5.32	3.30	2.02	1.63	0.174	163	202	+21
III. "Cobalt & Tur- pentine"	15.6	20.0	58.0	77	125	247	5.16	2.48	2.54	0.98	0.162	166	200	+20
IV. "Turpentine"	16.4	14.9	42.4	42	165	—	4.99	2.67	2.31	1.15	0.170	160	210	+31
V. "Cobalt after Turpentine"	15.9	17.0	47.2	—	—	—	—	—	—	—	0.159	165	217	+32

injected into the posterior muscle of the thigh. A definite abscess appeared by the third week.

In a third experiment the same procedure was followed but 14 rats were placed in each group and a fifth group was added (group V). The new group ("cobalt after turpentine") received the same preliminary treatment as group IV but, after anemia had developed, 0.125 mg. cobalt per rat was given daily. Thus the effect of cobalt on turpentine-induced anemia could be studied.

The first two experiments lasted 11 weeks. Since maximal polycythemia developed by the seventh week, the third experiment was terminated at 7 weeks.

Hemoglobin was determined by the photoelectric oxyhemoglobin method, using

an Evelyn photoelectric colorimeter. The instrument was standardized by the Van Slyke procedure and the hemin method of Clegg and King.<sup>15</sup> Blood was obtained from the tail. The plasma iron was measured according to the procedure of Kitzes, Elvehjem, and Schuette<sup>16</sup> in the first experiment and by the method of Barkan and

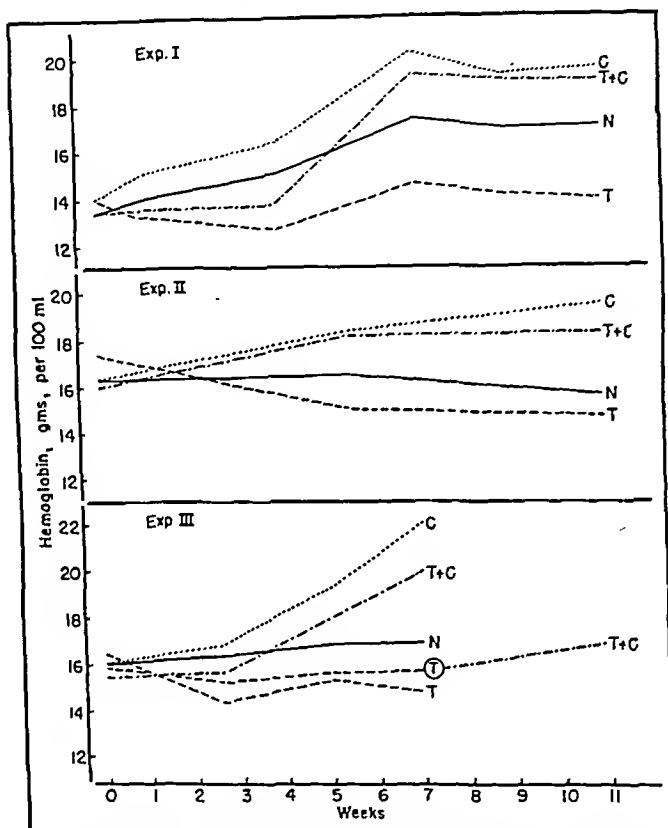


FIG. 1. HEMOGLOBIN LEVELS IN RATS INJECTED WITH TURPENTINE (T) COMPARED WITH NORMAL CONTROLS (N), THOSE GIVEN COBALT (C), AND THOSE INJECTED WITH TURPENTINE AND GIVEN COBALT AS WELL (T + C), IN EACH OF THREE EXPERIMENTS

In experiment III there was a fifth group of rats which was treated with cobalt after anemia had developed following the injection of turpentine.

Note that the administration of cobalt tended in large measure to overcome the effect produced by turpentine.

Walker<sup>17</sup> in experiments II and III. The method of Grinstein and Watson was followed for erythrocyte protoporphyrin determination.<sup>18</sup> Serum copper determinations were made by the method of Cartwright, Jones and Wintrobe.<sup>19</sup> Plasma proteins were determined by the biuret method of Kingsley<sup>20</sup> as modified by Weichselbaum.<sup>21</sup> The method of Van Slyke and Cullen<sup>22</sup> was used to measure the urea and ammonia of the urine.

At the end of the experiment the rats were anesthetized with nembutal and blood

was drawn from the abdominal aorta. Plasma iron, serum copper, erythrocyte protoporphyrin, and plasma proteins were determined on the pooled blood from each group of animals.

# RESULTS

The data obtained are summarized in table 1 and the more significant observations are illustrated in figures 1-4.

Figure 1 represents the result of hemoglobin determinations in the various groups in all three experiments. It will be seen that the results were essentially the same throughout. The administration of cobalt led to the development of polycythemia, and the injection of turpentine was associated with the development of a moder-

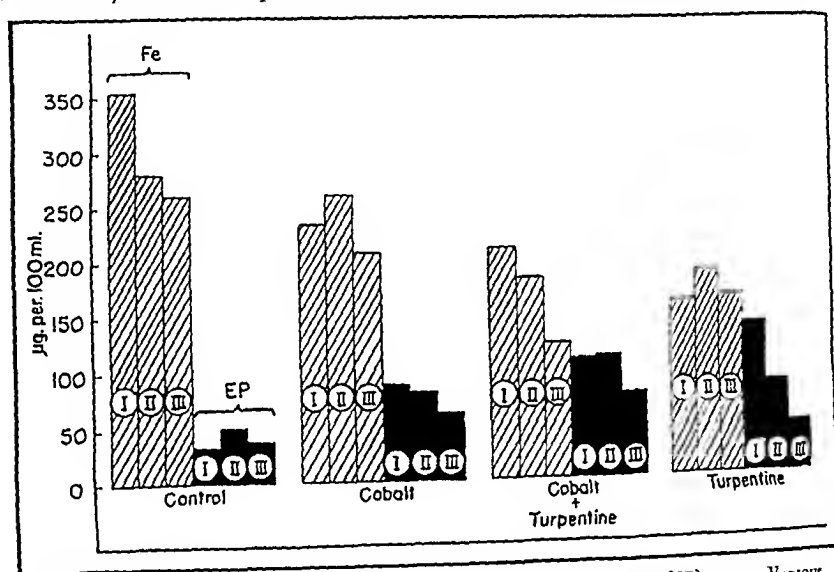


FIG. 2. PLASMA IRON LEVELS (Fe) AND ERYTHROCYTE PROTOPORPHYRIN (EP) IN THE VARIOUS GROUPS OF RATS IN EACH OF THE THREE EXPERIMENTS (I, II, III)

ate anemia. When cobalt was given in addition to turpentine, the tendency of the latter to produce anemia was overcome and polycythemia, somewhat less marked than that occurring in animals receiving only cobalt, developed.

The impairment of hemoglobin production which resulted from the injection of turpentine was associated with a reduction in the plasma iron level and an increase in erythrocyte protoporphyrin, just as has been observed in patients with anemia accompanying infection.<sup>2</sup> The results were similar in all three experiments (fig. 2). Considering the data for all three experiments, it would not seem that the simultaneous administration of cobalt and turpentine made a significant difference in the degree of hypoferremia produced, even though the impairment in hemoglobin production was largely overcome. The degree of increase in erythrocyte protoporphyrin appeared, on the whole, to be related inversely to the degree of hypoferremia. It is of interest that the administration of cobalt alone tended to be

associated with the development of somewhat lower levels of plasma iron as compared with the controls, and there was at the same time an increase in erythrocyte protoporphyrin. Somewhat higher levels of serum copper were noted in association with the administration of cobalt and the injection of turpentine than were found

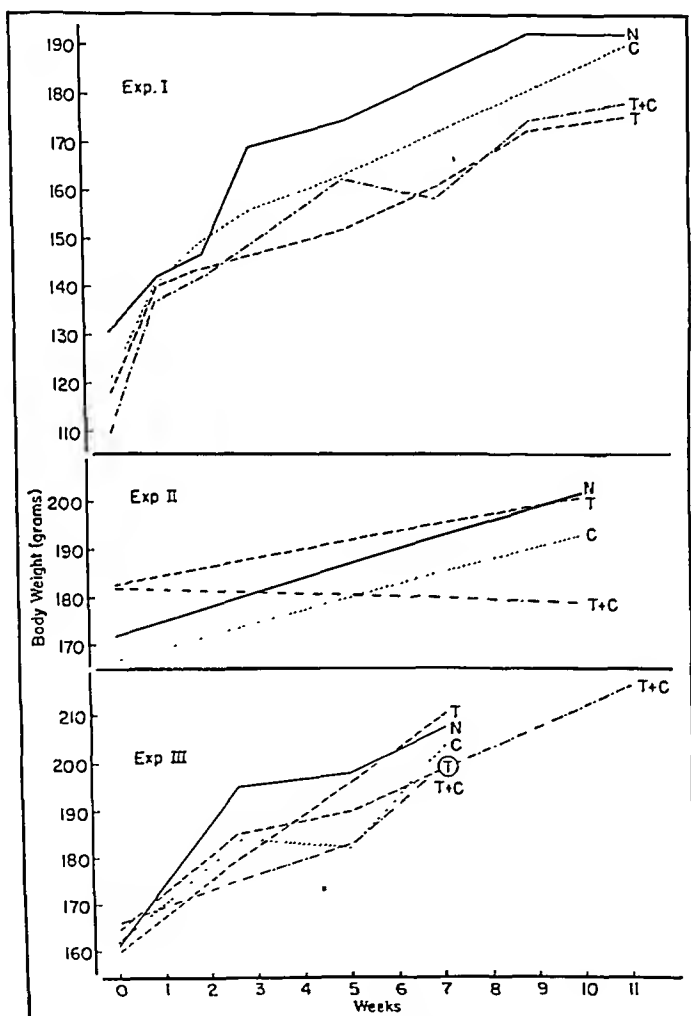


FIG. 3. GROWTH CURVES FOR ANIMALS IN THE VARIOUS GROUPS IN EACH EXPERIMENT

in the control animals but it remains to be determined whether these differences were significant.

Weight gain tended to be impaired both by the injection of turpentine and by the administration of cobalt, but was influenced more consistently by the latter. In experiment I the injection of turpentine was associated with the most pronounced impairment of weight gain, but in experiments II and III the "turpentine" groups

grew almost as well as the control animals while those receiving cobalt grew poorly. In all three experiments the rats receiving both turpentine and cobalt gained the least amount of weight. The data are illustrated in figure 3.

Total plasma protein levels were reduced in all the groups as compared with the controls, except for group II of experiment II. This was due to a reduction in the plasma albumin fraction. The data for experiment III, which we regard as the most reliable of those obtained in the three experiments, are illustrated in figure 4. Nitrogen excretion, as measured by the urine urea plus ammonia, was the same in all groups of rats.

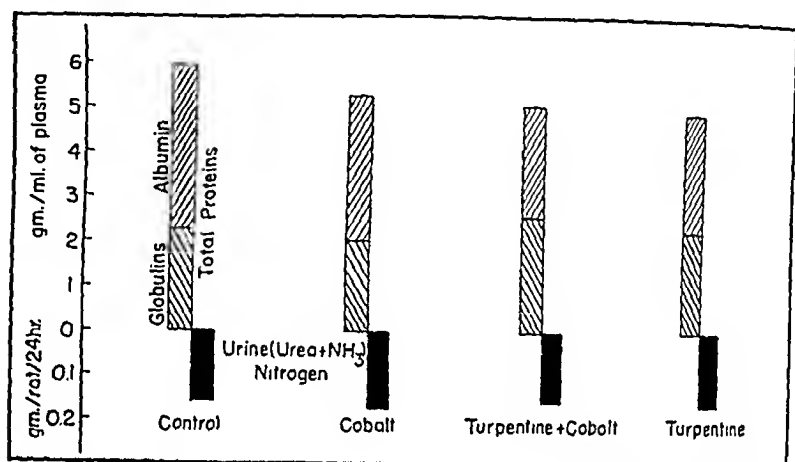


FIG. 4. ALTERATIONS IN SERUM ALBUMIN AND GLOBULIN AND TOTAL PROTEIN, AND URINE UREA PLUS AMMONIA EXCRETION IN EACH GROUP OF ANIMALS IN EXPERIMENT III

#### DISCUSSION

Since the anemia associated with infection is known to be refractory to therapy, it is of great interest that the administration of cobalt appears to overcome the retardation of hemoglobin formation which is produced by inflammation. Elucidation of the mode of action of cobalt might be expected to yield valuable information concerning the pathogenesis of the anemia of infection.

As yet, however, the mode of action of cobalt in polycythemia in normal animals is not known. It has been reported that the feeding of liver<sup>23</sup> or the injection of liver extract<sup>23,24</sup> or of ascorbic acid<sup>25</sup> tends to counteract or nullify the effect of cobalt. Cobalt did not produce polycythemia in splenectomized rats<sup>25</sup> or when the diet was deficient in iron or copper.<sup>27</sup> In fact, in the absence of copper, anemia developed.<sup>27</sup> When cobalt was fed prior to the addition of iron and copper, the normal response to such supplements given to dogs made anemic by hemorrhage was inhibited.<sup>28</sup> The report of Barron and Barron<sup>25</sup> that small amounts of cobalt inhibit the respiration, *in vitro*, of various tissues, notably of reticulocytes and bone marrow, could not be corroborated by Warren et al.<sup>29</sup> It was also demonstrated by the latter workers that the erythroid hyperplasia of bone marrow in cobalt-polycythemic animals is independent of whether or not the marrow has an intact pe-

ripheral innervation. They suggested as a mode of action of cobalt that there is some effect on the liver whereby the formation there of metabolic precursors requisite for red cell production is enhanced. That the action of cobalt may be based on a neural mechanism is an hypothesis arising from the reports of Davis<sup>30</sup> that choline and certain other vasodilator drugs depress or prevent the polycythemia which follows cobalt administration. Griffith et al.<sup>31</sup> have proposed that interference with cellular oxidation with the formation of complexes with sulfhydryl compounds, as for example with glutathione, may be the stimulus to the hemopoietic system which causes cobalt polycythemia. These investigators observed that methionine, cystine, and cysteine decrease the toxicity of cobalt. Since choline is metabolically related to the sulfur-containing amino acids, its counteracting effect on cobalt polycythemia might be explained in the same way. Orten and Orten<sup>11</sup> suggested that cobalt overcomes anemia due to protein deficiency in rats by making more available for hemoglobin synthesis the proteins of the "metabolic pool."

Kato and Iob<sup>32</sup> found that the spleen and bone marrow of dogs and rabbits fed cobalt in addition to iron contained less iron than those of animals given iron alone. This would suggest a more complete utilization of iron for erythropoiesis in the presence of cobalt. Whatever might be the means whereby cobalt accomplishes this, such an explanation is consistent with and could explain our own observations. Studies in this laboratory have shown that the hypoferrremia associated with infection is not due to a lack of iron and cannot be corrected by infusing iron intravenously<sup>33</sup> but must be assumed to be due to some disturbance related to iron metabolism whereby the utilization of iron is affected.<sup>34</sup> This fault would appear to be corrected in whole or in part by the administration of cobalt.

It is of interest that the plasma iron level of rats given cobalt (group II) was lower than in the controls and that the erythrocyte protoporphyrin was increased. The hypoferrremia is consistent with the assumption that hemoglobin synthesis is accelerated by cobalt. A rise in erythrocyte protoporphyrin is associated, according to Watson, Grinstein, and Hawkinson,<sup>35</sup> with normoblastic activity of the bone marrow, a feature which has been observed repeatedly in cobalt polycythemia.<sup>8,9</sup>

The observations cited in the present report would indicate that neither cobalt nor turpentine seems to increase protein catabolism, since no appreciable difference was found in urinary nitrogen (urea plus ammonia) excretion. The normal nitrogen excretion of the rats receiving only cobalt (group II) would support the opinion of several authors<sup>7,9</sup> that cobalt is not toxic in the doses ordinarily needed to produce polycythemia. The lower growth rate may be explained by a decreased food intake, since Frost et al.<sup>28</sup> claim that cobalt produces anorexia. Unfortunately, food intake was not measured in our animals.

It is proposed to repeat and extend in larger animals the studies reported here, since thereby it may be possible to carry out more detailed observations than are possible in the rat.

#### SUMMARY AND CONCLUSIONS

The influence of cobalt on the anemia associated with inflammation has been studied in three experiments involving observations in 108 rats.

It was found that by the simultaneous administration of cobalt the anemia asso-

ciated with inflammation, as produced by the injection of turpentine, could be prevented from developing and polycythemia appeared instead.

This effect was accompanied by hypoferrremia and an increase in erythrocyte protoporphyrin values similar to those encountered when anemia develops in association with inflammation.

Similar, though less marked, chemical changes were observed when only cobalt was given and polycythemia was produced.

A decrease in plasma albumin was noted in rats injected with cobalt or turpentine, or both, but this was not accompanied by an increased excretion of urinary nitrogen as measured by the urine urea and ammonia.

The observations cited are consistent with the hypothesis that cobalt favorably influences the utilization of iron for the synthesis of hemoglobin.

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## MONOCYTIC LEUKEMIA

### A CASE REPORT ILLUSTRATING VARIATIONS IN THE CLINICAL PICTURE

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THE subject of monocytic leukemia is beclouded with controversy and unsolved biological, cytological, and clinical problems. The full understanding and solution of these questions can be furthered by the recognition of the variegated features of this disease. Conversely, failure to appreciate this aspect leads to erroneous conclusions and apparently irreconcilable divergence of opinion. Thus, a survey of the literature uncovers a variety of interpretations that are frequently diametrically opposed. We believe that this paradox is, in part, due to failure to appreciate fully the natural history of monocytic leukemia.

Although most authors agree that monocytic leukemia is a distinct entity, this has not been universally accepted. Myelocytes and immature forms resembling myeloblasts may be found in monocytic leukemia and, conversely, the transient or persistent appearance of monocytes in myelogenous leukemia has been reported frequently. Naegeli,<sup>33</sup> Rosenthal,<sup>36</sup> and others deny the existence of monocytic leukemia per se and admit only a monocytic phase of myelogenous leukemia. Naegeli<sup>33</sup> believed the monocytosis in myelogenous leukemia is a "reactive monocytosis," while Hittmair<sup>32</sup> suggests that it is a "mesenchymal reaction." Campbell, Henderson, and Croom,<sup>5</sup> on the other hand, explain the myelocytic reaction during monocytic leukemia as an "irritative phenomenon (myeloid phase)" secondary to monocytic infiltration of the bone marrow. This antithetical argument, in which both sides utilize the same line of reasoning to support their views, stems primarily from the obscure origin of the monocyte and the lack of knowledge concerning its immature precursors (Jaffé<sup>24</sup>). Thus, in 1934, only 21 years after the initial report of monocytic leukemia by Reschad and Schilling-Torgau,<sup>35</sup> Forkner<sup>18</sup> could enumerate 19 views as to the probable source of the monocyte.

The theories concerning this cell's origin fall into three main groups. Naegeli<sup>33</sup> supports his concept that monocytic leukemia is a form of myelogenous leukemia by denying the existence of the monoblast and by declaring that the cell in question is a variant of the myeloblast. This cell he terms a para-myeloblast, a view which has secured considerable support. On the other hand, many authors believe that the monocyte can be traced back to one or more components of the reticulo-endothelial system (RES). Thus, either the reticulum cells or the endothelial cells individually have been incriminated by some writers, while others have felt that all parts of the RES can contribute to monocytopenesis.<sup>2,6,7,28-32,34,35,42-45</sup> The opinion is expressed frequently that the RES produces an immature cell, the hemohistioblast (which is identical with the monoblast), and that this cell subsequently

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develops into a monocyte. This immature cell is not identical with and does not resemble the other direct derivative of the RES, the histiocyte (macrophage).<sup>10,11,37</sup> Other writers prefer to consider a stem cell as precursor.<sup>8,27,41</sup> In a later publication, Doan and Wiseman<sup>14</sup> state that the general, loose, reticular mesenchymal connective tissue is the site from which the monocytes develop. To a certain extent this opinion is shared by Bock and Wiede<sup>2</sup> and Jaffé,<sup>24</sup> who believe that fibrocytes may develop into monocytes. Jaffé<sup>24</sup> considered this tissue to be an undifferentiated, blood-forming, germinal system which is not identical with the RES but which represents mesenchyma, a concept which concurs with the theory of Doan and Wiseman.<sup>14</sup> Campbell, Henderson, and Croom<sup>5</sup> also invoke the stimulation of a stem cell which differentiates either into monocytic or myelocytic cells.

An additional difficulty in securing a precise definition of monocytic leukemia is found in the indefinite descriptions of the histologic changes in this disease. Full use of supravital and differential staining technics has not been the rule. Jaffé<sup>24</sup> states that many authors do not differentiate between stem cells and monocytopoietic cells nor do they draw a distinct line between myeloid hyperplasia and metaplasia. He and others, for instance, have observed erythrophagocytosis in the peripheral blood in the monocytic phase of myelogenous leukemia, while DiGuglielmo<sup>12</sup> emphasizes that the cells of monocytic leukemia never contain red cells, cellular debris, or iron pigment; this is a typical lack of agreement which is reproduced so frequently in the literature.

While many authors state that monocytes and promonocytes are oxidase positive, there is no unanimity of agreement concerning this reaction. Foord, Parsons, and Burt<sup>17</sup> failed to observe a positive oxidase in 3 cases. Campbell, Henderson, and Croom<sup>5</sup> found the peroxidase reaction to be positive in only a minority of the cells; it was very fine and not the coarse, gross reaction seen in myelocytes. They thought that these cells resembled the blood histiocytes of Dameshek rather than true monocytes. Osgood<sup>31</sup> did not believe that the peroxidase is of any value in differentiating monoblasts from myeloblasts or lymphoblasts and felt that the oxidase-positive granules found in monocytes are, in reality, engulfed inclusions of degenerating granulocytic cells. Doan and Wiseman<sup>14</sup> considered the oxidase-positive granules in monocytes to be different in location and degree from those seen in the granulocytic cells.

It should be pointed out also that various authors admit the considerable difficulty encountered in distinguishing and identifying the various cells under discussion. Merklen and Wolf<sup>23</sup> state that the only certain way they could identify the monoblast was by the presence of large numbers of monocytes. Isaacs<sup>23</sup> thought that finding all gradations between monoblasts and monocytes was helpful in identifying the various cells. The fact that monocytic leukemia is a relative newcomer to the leukemias also indicates the difficulty in cytologic differentiation.

To establish some degree of order in this otherwise chaotic and contradictory mass of information, Gittens and Hawksley<sup>19</sup> presented a classification of leukemic monocytosis based on the segregation into one group of those cases showing specific involvement of the RES or cases showing a generalized monocytic infiltration; the cases of monocytosis with the histological features of myelosis were placed in

another group. Using as their criteria the characteristic alterations of the reticulum in relation to the predominating blood cell, Campbell, Henderson, and Croom<sup>1</sup> classified reported cases into those showing (a) hyperplastic reticulum without blood monocytes; (b) hyperplastic reticulum with increase in blood monocytes, and (c) no particular tissue or reticulum formation but demonstrating purely blood cell differentiation into monocytes and granulocytes.

The most popular classification at the present time is that of Downey,<sup>15</sup> who divides monocytic leukemia into two main groups. The differentiation is based on (a) the derivation of the predominant cell, (b) the distinguishing characteristics of this cell, and (c) the histopathologic changes which occur in the blood-forming organs. The first group he terms the Naegeli type, since it is considered a variant of myelogenous leukemia, a view first expressed by Naegeli, who favored a myeloblastic origin of the monocyte. In this form transitional stages between myeloblasts and monocytes may be observed in blood and bone marrow. Often this type terminates without significant alterations in the blood picture. At autopsy, infiltration of large mononuclear cells unassociated with hyperplasia of the reticulum can be found in the hematopoietic organs. A small percentage of cases terminate with the picture of myelogenous leukemia with characteristic changes in the blood-forming organs.

The second, or Schilling type, on the other hand, shows transitions between cells of the RES and monocytes. This is associated with systemic and excessive hyperplasia and proliferation of the reticular tissue in blood-forming organs, with differentiation into monoblasts and the appearance of reticulo-endothelial cells, monoblasts, and monocytes in the blood. Because of this relationship, the terms "reticulo-endotheliosis," "reticulosis," and "leukemia reticulo-endotheliosis" have been used synonymously with monocytic leukemia by some authors, while others distinguish "leukemic or aleukemic reticulo-endotheliosis" from "nonleukemic reticulo-endotheliosis." Downey,<sup>15</sup> Osgood,<sup>31</sup> and others believe that these terms should not be used to denote monocytic leukemia.

Baserga<sup>3</sup> introduces a line of reasoning in this problem which perhaps may serve to breach the schism. He states that the appearance of an erythroblast in myelogenous leukemia does not make this an erythro-myelogenous leukemia; therefore, the presence of myelocytes in monocytic leukemia does not necessitate the diagnosis of monocytic-myelogenous leukemia. It is still monocytic leukemia.

The hematologic differentiation of the Schilling type may be quite difficult from those instances of symptomatic, transient monocytois due to sepsis lenta, in which an increased number of monocytes and hyperplasia of the RES are found. In instances of reticulo-endotheliosis due to intoxications and granulomatous reactions (syphilis, tuberculosis, Hodgkin's disease, and lupus erythematosus), a marked leukemoid monocytic reaction may be encountered. Doan and Wiseman<sup>14</sup> have indicated a possible relation between the monocytois in Hodgkin's disease and that of monocytic leukemia. Ewing<sup>16</sup> states that many authors have associated monocytic leukemia with tuberculosis. Rosenthal and Abel<sup>33</sup> have indicated an allied phenomenon—leukopenic infectious monocytois sometimes seen in agranulocytosis.

The literature depicting the clinical features is likewise contradictory. Forkner,<sup>18</sup> Dameshek,<sup>10,11</sup> Mitchell,<sup>30</sup> Osgood,<sup>34</sup> Campbell, Henderson, and Croom,<sup>5</sup> and others have stressed the striking frequency of tumefaction of the gums and the presence of ulcerative mouth lesions in monocytic leukemia. On the other hand, Watkins and Hall,<sup>45</sup> Wintrobe,<sup>47</sup> Jaffé,<sup>24</sup> and others do not regard these gum changes as characteristic or exclusively representative of this disease. The data of Watkins and Hall<sup>45</sup> may clarify this divergency, however, inasmuch as 3 out of 9 acute, and 2 out of 14 chronic Naegeli types showed gum involvement in contrast to the complete absence of this change in all of the 6 patients of Schilling type. Doan and Wiseman<sup>14</sup> originally believed that there is no clinical syndrome which is pathognomonic of monocytic leukemia and which can differentiate it from other types; however, recently they<sup>13,48</sup> have reversed their previous stand and feel that gum changes are typical of this disease. Watkins and Hall<sup>45</sup> affirm that there is no essential clinical difference between this and other leukemias.

The course of the disease is usually acute. Jaffé states that "true chronic monocytic leukemia has not yet been described, and I think that when a case of monocytic leukemia lasts long enough, it sooner or later turns into a myelosis." Naegeli also believes that "it [monocytic leukemia] is a temporary variant of myelogenous leukemia, into which it passes unless death intervenes." In Osgood's<sup>34</sup> series of 147 cases, 77 per cent (acute group) lived six months or less, 13 per cent existed from six months to one year (subacute group), and only 10 per cent (chronic) lived more than one year. In Watkins and Hall's<sup>45</sup> series there were 23 of the Naegeli type, with duration from five weeks to six years, while the 6 Schilling types lived from seven weeks to twenty-seven months.

Sex predilection in this disease seems to be agreed upon. Osgood<sup>34</sup> noted that over two thirds of his cases occurred in males, a relationship which has also been mentioned by others. There is some tendency to remission in acute monocytic leukemia, as Campbell, Henderson, and Croom<sup>5</sup> have indicated. The incidence of this disease is calculated at 3 per cent to 9 per cent of all leukemias by Osgood,<sup>34</sup> and Rosenthal and Harris<sup>10</sup> suggest that this relative incidence is about that of the various types of cells in normal blood.

The differential diagnosis of monocytic leukemia, especially in the subacute stage, where atypical cases are more likely to be found, poses many difficulties.<sup>46</sup> The group of cases displaying a normal or leukopenic white count and showing atypical, immature forms of monocytes, nucleated erythrocytes, and myelocytes requires intensive study and prolonged observation for eventual clarification.<sup>25</sup> The degree of anemia has been noted frequently to be more severe<sup>1</sup> and hemorrhagic phenomena are more likely to occur<sup>46</sup> in such cases than in typical instances of chronic or subacute leukemia of other types. The differential diagnosis between monocytic leukemia and agranulocytosis, purpura, aplastic anemia, splenic neutropenia, splenic panhematopenia, and other leukemias often will depend solely upon the results of bone marrow examinations. The presence of "reactive" or "irritative" phenomena, as mentioned above in connection with "mixed" types, or the leukemoid reaction in those nonleukemic states of inflammation or intoxications, must be considered and evaluated in the differential diagnosis.

In the last analysis, the diagnosis must be established by the morphological characteristics of the predominating cell as revealed by the fullest utilization of all applicable laboratory technics. These should include daily, complete blood counts, examinations of fixed and supravital stained smears of the peripheral blood, and frequent bone marrow aspirations. Biopsy of lymph node and skin nodules frequently offers valuable information, especially when Giemsa, azure-eosin, and reticulum stains are used. The oxidase test should be employed in fresh smears and frozen fixed tissues. Darkfield observation<sup>7</sup> often clarifies the position, degree, and type of cytoplasmic granules within the monocytoïd cell. We do not agree with Hall<sup>21</sup> or Bloom<sup>4</sup> as to the unreliability of the supravital technic in identifying these cells, although we agree that one should not attempt to establish the diagnosis by this single technic alone.

The purpose of the foregoing discussion has been to indicate the difficulty in delineating the clinical syndrome and the cytologic characteristics of monocytic leukemia. Aside from conflicting theoretical concepts, some of the confusion is due to incomplete reports in which necropsy descriptions are lacking or are sketchy, and the histologic analysis has failed to utilize the differential staining technics described above. In addition, continuity of observation of this disease, from onset of symptoms to eventual death, has often been lacking and, furthermore, the impression is gained that some observations are not as devoid of preconceived convictions as is desirable. We believe, as we shall illustrate by the case to be presented, that prolonged, intensive, clinical and laboratory observation will lead to an appreciation of the vagaries of this disease. Such observations have led us to the belief that some of the discrepancies in the literature can be synthesized into an organically unified and acceptable concept.

#### CASE REPORT

This 18 year old white male had been under intermittent observation for over nine months (because of headaches resulting from repeated head injuries) before the initial manifestations of his eventually fatal illness became apparent. Figures 1-3 and table 1 chronologically present the voluminous clinical and laboratory data in graphic form.

In November 1943 the blood count was normal. At that time the headaches were found to be due to traumatic encephalopathy. Four weeks later the patient contracted an apparently simple upper respiratory infection. Within 24 hours he was acutely ill and febrile and he developed cervical adenopathy and a palpable spleen. A blood count revealed a relative leukopenia and severe granulocytopenia.

No etiologic factor could be ascertained as responsible for this granulopenic state. Pentnucleotide was administered for seven days in adequate dosage but the illness progressed alarmingly with increasing toxicity, fever, tachycardia, pharyngitis, tonsillar ulceration, hyperplastic gingivitis, regional adenopathy, and splenomegaly. On the fourth day of illness, penicillin therapy was instituted, the patient receiving 25,000 units intramuscularly every three hours. Clinical improvement was evident in the next 24 hours, although he developed an additional tonsillar ulcer and a harassing cough. In 48 hours the patient was no longer critically ill; the ulcerations began to heal and the fever diminished. Penicillin was administered for a total of ten days and was finally discontinued when all signs and symptoms disappeared.

During this remission the patient was asymptomatic, afebrile, and revealed no abnormalities on physical examination. Blood counts, however, disclosed slowly progressive anemia and leukopenia. Thus, the second attack of agranulocytosis, of 18 days' duration, was established by laboratory studies 9 days prior to the onset of signs and symptoms. On the 37th day of illness (January 25, 1944) sepsis was

evident. There were fever, early gingivitis, mild pain in the neck and jaw, and rectal discomfort from perianal inflammation. Increasing pharyngitis, cervical adenopathy, and trismus soon became evident. Intramuscular penicillin therapy was resumed on the 39th day and the patient received a total of 160,000 units daily. At this time the gingivae were inflamed and hyperplastic so that the teeth were partially buried, an erosion appeared at the angle of the mouth, there was a necrotic ulceration behind a left lower

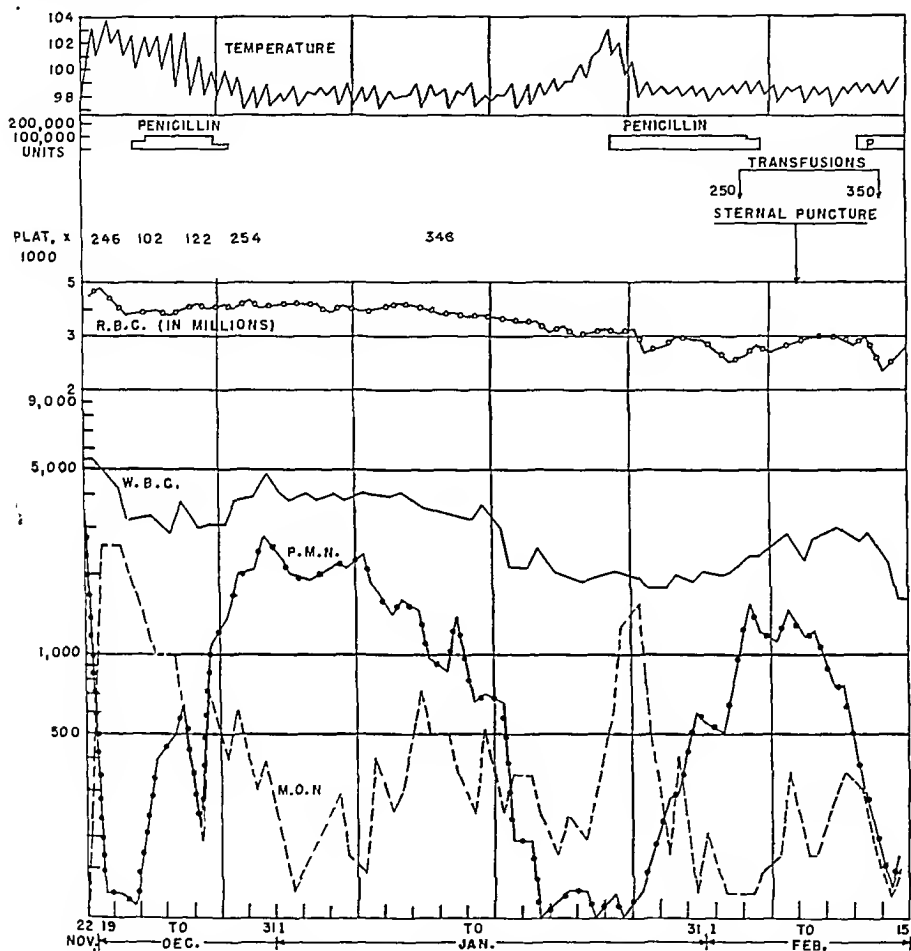


FIG. 1. COMPOSITE GRAPH, PLOTTED ON SEMILOGARITHMIC PAPER, OF THE HEMATOLOGIC AND CLINICAL OBSERVATIONS FROM NOV. 22, 1943, TO FEB. 15, 1944

Red blood cells (RBC); white blood cells (WBC); polymorphonuclear granulocytes (PMN); monocytes (Mon); immature monocytes (Immat).

molar tooth, and the perianal inflammation had spread extensively. After 48 hours of penicillin therapy, in the presence of a maximum white blood count of 1,800 with 4 per cent granulocytes, there was striking clinical improvement; the temperature did not rise above 100.6 degrees F., and there was notable recession of the mouth lesions and the perianal inflammation. Despite the fact that the white blood count did not rise above 2,000 cm. with 22 per cent granulocytes, the mouth and anal lesions healed promptly although the lip erosion required another week for epithelization.

The first sternal marrow aspiration was performed after this second agranulocytotic episode on Febru-

ary 7, 1944, the 54th day of illness (table 1). The peripheral blood and sternal marrow smears were submitted to several consulting hematologists. All opinions were conflicting and differed in the classification and interpretation of the cells of the mononuclear group. The majority opinion was that the findings were suggestive of, but not conclusive for, leukemia.

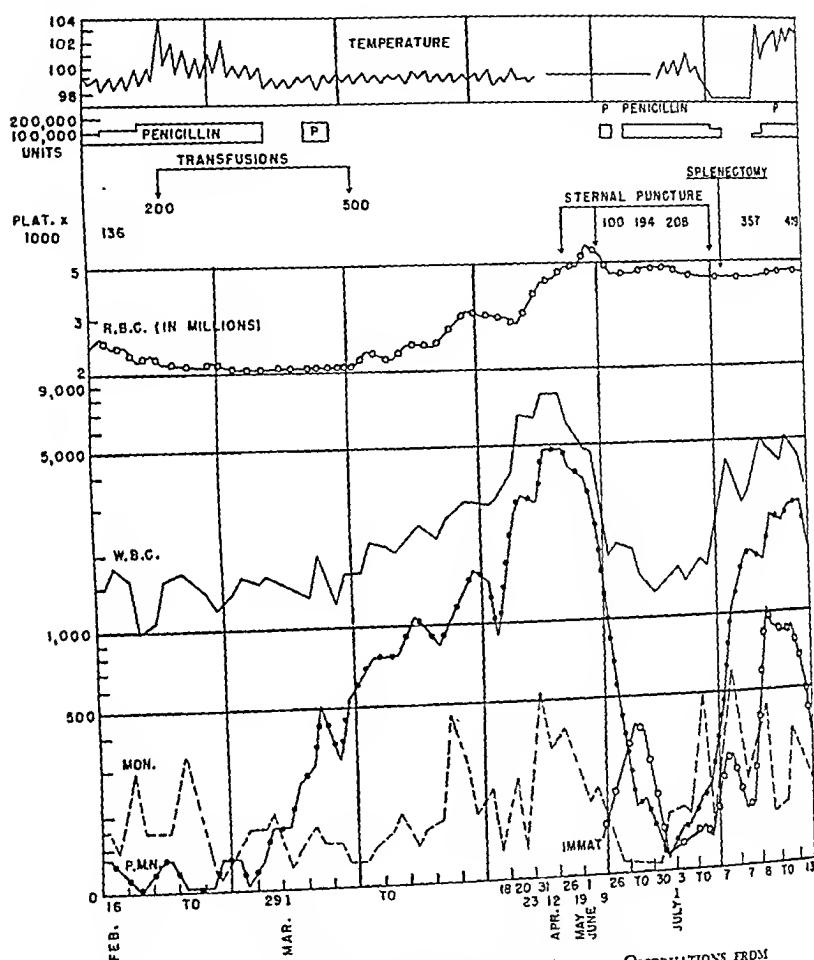


FIG. 2. COMPOSITE GRAPH OF HEMATOLOGIC AND CLINICAL OBSERVATIONS FROM FEB. 16, 1944, TO JULY 13, 1944

The third and longest period of granulocytopenia began on the 58th day of illness (February 11). Similarly, it was detected initially by the serial blood counts. For 20 days the total circulating granulocytes were never greater than 225 per cm., and they usually ranged around 75 per cm. For five days no granulocytic cells could be found in the peripheral blood. Because of the hematologic warning of impending sepsis, penicillin therapy was instituted prior to the development of signs and symptoms, the patient receiving a total of 2,820,000 units in a period of 21 days. No other medication was employed, and for 10 days the only abnormalities detected were a single temperature reading of 100.0 degrees F., a thermal reaction due to a transfusion, a mild hyperplastic gingivitis, a soft apical systolic murmur, and the development of a palpable liver and spleen two finger-breadths below the costal margins.

The prognosis was deemed poor at this time (February 27, 1944) in view of the weakness, marked

weight loss, and the progressive development of an aplastic anemia syndrome. From the 80th to the 119th day of illness, however, there was a spectacular improvement in the hematologic status. Blood smears showed a classical "leukemoid" reaction and all blood elements returned to normal limits rapidly associated with recovery of weight, strength, and sense of well-being. A sternal marrow aspiration was performed and it was reported that the previous provisional diagnosis of leukemia could not be substantiated.

There followed a clinical and hematologic remission of approximately three months' duration, during which time the patient was transferred to a hospital near his home where he was again under the obser-

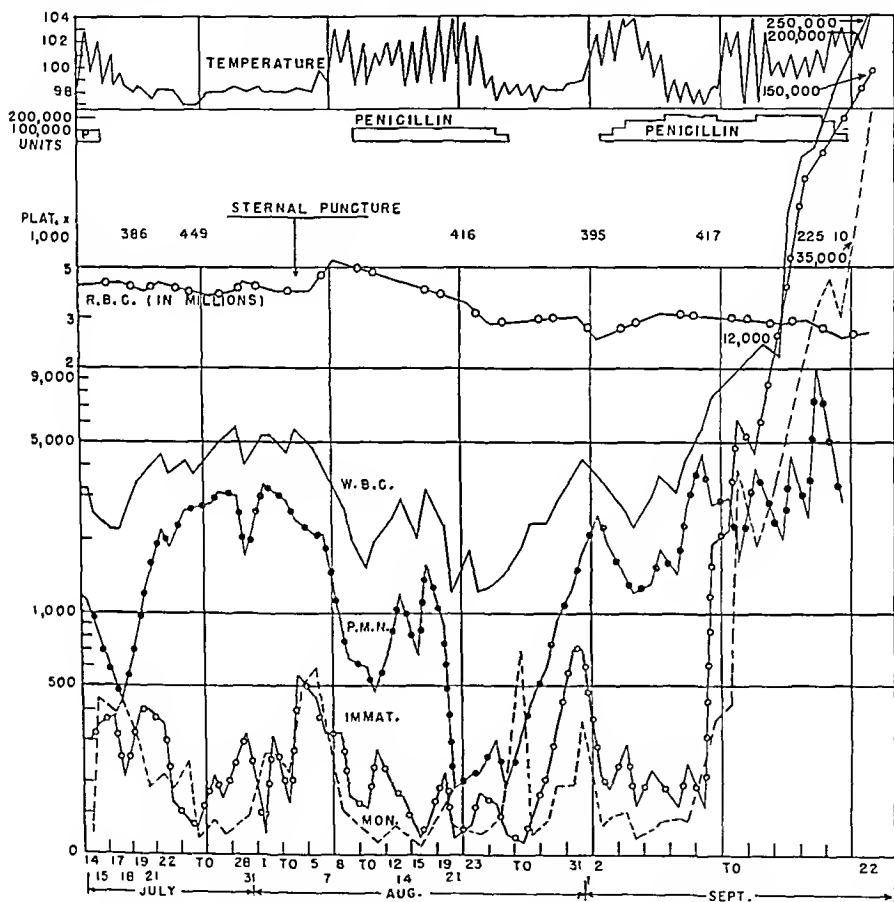


FIG. 3. COMPOSITE GRAPH OF HEMATOLOGIC AND CLINICAL OBSERVATIONS FROM JULY 14, 1944, TO SEPT. 22, 1944

vation of the same medical officer (VHK). The previous suspicion of leukemia could not be established by the admission studies.

A fourth episode of granulocytopenia began on June 26 and was treated promptly and exclusively with penicillin. Mild fever was the only clinical indication of possible infection. In view of a nondiagnostic sternal aspiration, a nondiagnostic thrombocyte count, the presence of phagocytosis of red blood cells and possibly of white blood cells, and the general nonconformance to a leukemic picture, consideration of splenic neutropenia (Doan and Wiseman<sup>14</sup>) was entertained. An exploratory laparotomy was performed for the purpose of securing diagnostic material. No abdominal adenopathy was observed; the liver was

TABLE 1.—*Summary of Bone Marrow Studies*

Cells	Normal Range	Found					
		2/8/44	4/26/44	6/22/44	7/6/44	8/4- fixed	8/4- ravil.
Mycloblasts.....	0.3 - 5.0						
Promyelocytes.....	1.0 - 8.0	0.8	0.3	3.0	12.0	1.0	
Myelocytes: A).....				27.0	10.5		
B).....						4.0	1.0
C) Neutrophilic.....	5.0 - 19.0	4.2	3.6	3.5	4.5	1.0	0.5
Eosinophilic.....	0.5 - 3.0						
Basophilic.....	0.0 - 0.5					9.0	11.0
Metamyelocytes (juvenile forms)....	13.0 - 32.0	10.4	13.0	4.5	5.5	4.5	0.5
Polymorphonuclear leukocytes.....	7.0 - 30.0	18.2	44.0	18.5	2.5	6.0	3.5
Polymorphonuclear eosinophiles.....	0.5 - 4.0		1.3	0.5	0.5	1.5	0.5
Polymorphonuclear basophiles.....	0.0 - 0.7					1.5	
Lymphocytes.....	3.0 - 17.0	17.6	20.0	20.0	48.5	20.0	13.0
Plasma cells.....	0.0 - 2.0		1.6				
Monocytes: Typical ...	0.5 - 5.0	16.0		1.0	0.5	16.5	29.0
Atypical (immature).....		16.8				25.5	30.5
Reticulum cells (stem cells).....	0.2 - 2.0	5.6	2.6				
Megakaryocytes.....	0.03 - 3.0			1.0		0.5	
Pronormoblasts (macroblasts).....	1.0 - 8.0			1.5	2.0		
Normoblasts.....	7.0 - 32.0	11.0	11.3	16.0	10.0	9.0	10.5
Megaloblasts.....				2.0	1.5	1.0	
Erythroblasts.....				1.5	1.5	1.0	

A

B

C

D

FIG. 4. SMEARS OF THE PERIPHERAL BLOOD IN THE TERMINAL STAGE, SHOWING THE VARIATION IN APPEARANCE OF THE MONOCYTIC CELLS

The lobulation and grooving of the nuclei, and the granulation of the cytoplasm are clearly demonstrated, while the irregular size and shape of the cells is evident. ( $\times 1515$ .)

not unusual, but because of its increased size the spleen was removed. The results of the histologic examination of this organ are included in the autopsy report.

There was clinical and hematologic improvement for a short period after splenectomy. The white cell count rose to normal limits but significant numbers of immature monocytes appeared. After eight days, however, granulocytopenia recurred and penicillin was again administered. Although no overt signs of sepsis appeared, the course was now febrile and remained irregularly so until death.

In addition to loss of weight and strength, there were complaints of bone pain, mainly in the sternum and ribs, and left flank pain. Neither x-rays of the bones nor a complete genito-urinary survey yielded positive findings.

A fifth episode of granulocytopenia, without mucocutaneous lesions, occurred in the latter part of August 1944 and was again treated with penicillin, but with no effect on the elevated temperature. A progressive leukocytosis appeared, which eventually reached 280,000  $\text{cm}^3$ , composed mainly of atypical monocytes and monoblasts. During this terminal phase there were severe vomiting, complete anorexia, constant pain in the ribs and sternum, abdominal pain, fever, tachycardia, and psychosis. An electrocardiogram obtained 20 minutes prior to death on September 22, however, revealed only tachycardia and very low voltage of all waves. Death occurred from acute circulatory failure nine and one-half months after the onset of the illness.

#### HEMATOLOGIC FEATURES

Analysis of the hematologic data requires precise cytologic descriptions for an exact definition of the monocytoid cells concerned in this disease. The following characteristics are based on daily examinations of fixed and supravitality stained smears of peripheral blood and of periodic bone marrow aspirations over a period of nine and one-half months (fig. 4).

The *monocyte* is usually larger than the granulocyte; its cytoplasm is opaque, mottled, gray-blue, and contains azurophilic granules. The cell margins are serrated and irregular. The nucleus is reniform or horseshoe-shaped and possesses a lacy, skein-like, coarse chromatin weave. "Grooving," "crumpled appearance," "lobulation," "indentation," and "folding upon itself" are characteristic terms used in denoting this peculiar configuration. With supravital stain, numerous neutral red or salmon-pink colored bodies, vacuoles, are symmetrically arranged around a centrosphere located in the bay (Hof) of the nucleus. This structure is the rosette which is considered so typical of the mature monocyte. Green-tinged mitochondria are small, fine, and few, and are located peripherally to the rosette and nucleus. The cell's motility is very typical and specific and is best described as a "sliding" or "gliding" motion, the cytoplasm flowing extremely sluggishly and the contour of the cell being wavy or undulating.

The *monoblast* measures from 15 to 20  $\mu$  in diameter. In fixed, stained smears it possesses a basophilic cytoplasm containing no granules and, distinctively, no Auer bodies. The nucleus is round, large, and centrally situated; its chromatin is fine, stippled, and sieve-like, and usually possesses two nucleoli. In supravitality stained preparations, motility, neutral red bodies, rosettes, and vacuoles are absent.

Between these poles of maturity, one notes a group of cells demonstrating a variable degree of differentiation. The cytoplasm displays a lesser degree of basophilism than the monoblast and occasional azurophilic granules are present. The nucleus is coarser than that of the monoblast but, in contrast to the monocyte, discloses a finer and more lacy chromatin network. Occasionally nucleoli are observed. The nucleus shows early indentation of its rather sharply delineated membrane. With supravital stain, neutral red bodies are present, although they are much finer and

more uniform in size and shape; they are more diffusely arranged throughout the cell and do not form the typical rosette of the mature monocyte. Motility is present at times but it is less active and often is not present. Occasionally in both fixed and supravital stained preparations, erythrophagocytosis is detectable. This intermediate form, which can be termed the *promonocyte* or *immature monocyte*, shows no transitional developmental forms to cells of the myelocytic series, and its relationship to the monoblast and the monocyte can be readily demonstrated. The presence of this intermediate atypical group has been mentioned previously by Osgood<sup>14</sup> and Downey.<sup>15</sup> A noteworthy characteristic of the promonocytic cell observed in this case is its unusual shape, since it may be elongated, tailed, grotesquely lobulated, or forked.

The qualitative and quantitative relationships of the monocytoid cells to the other blood constituents may best be demonstrated by inspection of figs. 1-3. For the purpose of analysis of this data, we have arbitrarily divided the discussion into two phases; namely, the initial period from the onset of the disease (December 1943) to the first prolonged remission (April-June 1944), during which time the clinical and hematologic diagnosis remained in doubt; and the second period, following the remission, during which the frank picture of monocytic leukemia finally emerged.

During this first period, the red blood cells underwent a progressive reduction in numbers, falling to a low of 1,700,000 cm. in February 1944, which continued for approximately six weeks. Erythropoiesis was heralded by the appearance of large numbers of nucleated red blood cells in the peripheral blood and by polychromasia and macrocytosis.

The granulocytes during the initial period underwent three distinct episodes of granulocytopenia, accompanied by an absolute increase in monocytic cells. This monocytosis prevented the fall of the total white cell count to a degree commensurate with the granulocytopenia. Similar to the erythropoietic reaction mentioned above, the remissions of granulocytopenia were introduced by evidence of granulopoiesis. Particularly in the recovery phase of the third episode of neutropenia, a very pronounced myeloid reaction was observed which was characterized by the appearance in the peripheral circulation of large numbers of myelocytes and promyelocytes.

Simultaneously with the ebb and rise of granulocytic cells, increased numbers of mature and immature monocytes appeared in the peripheral blood; these, intermingled with the myeloid elements, created a bizarre and confusing picture. The separate identity of both myeloid and monocytic cell types could be determined in later examinations by means of supravital stained smears. There appeared to be no transition between the monocytic and granulocytic series. The supravital technic seemed particularly valuable in securing evidence to demonstrate the relationship between the monocyte and the "blast forms." The latter were present in large numbers and their identification as to whether they were monoblasts or myeloblasts was difficult. During the recovery phase of the third episode of granulocytopenia there was a progressive reduction of all monocytic cells.

The thrombocyte count disclosed moderate transient reduction in numbers on

repeated examinations. While the tourniquet test was moderately positive, there was only slight tendency toward purpura.

Following the remission of about three months' duration, the hematologic equilibrium was once again disturbed by the development of profound anemia, leukopenia, granulocytopenia, and the appearance of an abnormal number of mature and immature monocytes. After splenectomy there was a transient rise of the white blood cells. Attention at this time became focused on the disquieting behavior of the monocytoid cell, which now began to increase in numbers and to reveal evidence of immaturity and atypism. The previous hesitancy and uncertainty over the clinical diagnosis and hematologic classification disappeared as the clear-cut pattern of monocytic leukemia emerged. The terminal phase of the second period provided a dramatic scene of tidal flooding of the peripheral circulation by monocytic cells, with the white cell count attaining a total of 280,000 cells per cm.

This frankly leukemic reaction was not associated with striking myelocytosis, the granulocytic cells being mature and well differentiated. The clear-cut relationship of monocyte to monoblast through an intermediate promonocytic stage assured the accuracy of the identification of the "blast" cell. This evidence supplied confirmation of the tentative designation of "monoblast" to these cells in the first phase of the disease.

Table 1 reveals the findings of repeated sternal marrow aspirations.

The first aspiration was performed on February 7, 1944, after the patient had experienced two episodes of granulocytopenia. Owing to the marked increase of monocytes in the peripheral blood, there was considerable doubt as to the correctness of the diagnosis of agranulocytosis, although leukopenic infectious monocytosis (Rosenthal and Abel<sup>38</sup>) was considered. The marrow aspiration disclosed almost 33 per cent mature and immature monocytes and an increased number of myeloid elements. These findings, in addition to the other hematologic data, seemed to support a diagnosis of monocytic leukemia (Naegeli type). The resident hematologist was convinced that the "blast" cells encountered in such large numbers were of the monoblastic variety rather than the myeloblastic. On consultation, two other hematologists confirmed the predominance of "blast" forms, but both preferred to label them myeloblasts. While one consultant seemed in favor of a diagnosis of myeloid leukemia, the other consultant thought that the entire picture might fit either a monocytic leukemia or a myeloblastic leukemia.

The difficulty in distinguishing myeloblasts from monoblasts has been pointed out. In this case, however, the high percentage of mature and immature monocytes in the bone marrow offers a definite clue in identifying the "blast" cells, inasmuch as derivation of the former from the latter must have occurred in order to explain the presence of monocytes in an organ where they are normally rarely found. Thus, the presence of a maturation gradient of monocytic elements from the earliest stage to adult forms should permit the identification of the "blast" cells as monoblasts. We feel that the consultants, when they arrived at a diagnosis of myeloblastosis, neglected to evaluate the presence of the mature monocytes.

The second aspiration was performed two and one-half months later, at the height of the hematologic remission. The values of the various cellular constituents

of the marrow now approximated the normal range and the "previous provisional diagnosis of leukemia could not be substantiated."

The third aspiration was carried out on June 22. The findings of this examination were interpreted as indicating a maturation arrest of the granulocytic cells inasmuch as there was an inordinate increase of promyelocytes. Four days later the fourth precipitous decline in total and granulocytic cells in the peripheral blood occurred. It is noteworthy that the bone marrow alterations preceded the changes in the peripheral circulation by an appreciable interval and heralded an impending leukopenia which was otherwise not apparent.

The fourth bone marrow examination, three weeks later, featured an even more marked reduction in the number of myelocytes and mature granulocytes. "Blast" cells were also increased and they were classified at this time as myeloblasts on the basis of their morphologic characteristics, since, unlike the initial sternal marrow examination, there was no accompanying monocytosis. Fig. 2, however, discloses the presence of large numbers of mature and immature monocytes in the peripheral blood, while analogous cells are conspicuously absent in the sternal marrow. Unless this absence were focal and not widespread throughout the bone marrow, it would appear that extramedullary monocytopenia was occurring in order to supply the peripheral blood with these elements. Similarly, myeloid metaplasia might be assumed to exist in foci other than the bone marrow.

The final bone marrow aspiration was performed about one month later, when the peripheral blood possessed its normal complement of white cells and granulocytes. In addition to this, the monocytes were markedly increased and comprised both mature and immature forms. The bone marrow picture at this time revealed an unequivocal monocytosis and granulocytopenia. These findings were considered diagnostic of monocytic leukemia, a conclusion subsequently confirmed.

#### ABSTRACT OF AUTOPSY PROTOCOL

*General.* The body was that of a 28 year old, somewhat emaciated, white male. Generalized lymphadenopathy was absent. The surface of the radix mesenterii was diffusely studded with small, dry, lusterless, firm, white-gray plaques possessing central, cherry-red areas. Many small, discrete lymph nodules could be felt in the intermesenteric folds.

*Lungs.* The lungs contained firm, circumscribed, moist nodules throughout.

*Thymus.* The thymus, markedly enlarged, weighed 65 grams. It was soft and contained many circumscribed firm nodules.

*Heart.* The pericardium and the myocardium were involved by diffusely scattered, irregularly sized, gray-pink plaques which extended into the subendocardial layer and the papillary muscles (fig. 5).

*Spleen.* Splenectomy was performed on July 7, 1944. Immediately after ligation and removal, the spleen weighed 468 grams. The organ was boggy, and its cut surface was moist, dark-red brown.

*Liver.* The liver weighed 2,400 grams and was enlarged. The surface was mottled by areas of yellow-gray which were distributed irregularly in a background of dark brown.

*Gastrointestinal tract.* The mucosa of the stomach contained discrete nodular masses. Several peripyloric lymph nodes of gray-white color were present. Peyer's plaques and solitary follicles were moderately enlarged.

*Kidneys.* The parenchyma was hazy and of light yellow hue. In the peripelvic fat of the left kidney there was a bean-sized, poorly demarcated mass of indurated tissue which possessed a gray-yellow, moist surface.

*Pancreas.* At the tail of the pancreas, in the region of the pedicle of the spleen, there was a poorly demarcated, plum-sized mass of red, moist tissue. At the site of ligation a mass of white, somewhat caseous necrotic tissue was present, in which suture material might still be recognized.

Bladder, testicles, prostate, brain, and adrenals revealed no gross abnormalities.



FIG. 5. PHOTOGRAPH SHOWING MULTIPLE PLAQUES OF MONOCYTIC INFILTRATION THROUGHOUT ALL LAYERS OF THE HEART

Bone marrow (sternum, vertebra, and tibia) was gray, red, and of mush consistency.

*Microscopic Examination of Tissues.* The common denominator of the changes in all organs was the presence of mononuclear cells. The organs were involved by these cells either by thrombosis of the blood vessels with resulting circulatory changes or by their infiltration in the interstitial tissues. The latter process led to architectural alterations which were either circumscribed or diffuse, causing compression atrophy, or necrosis of the native cellular components of the organ.

The prevailing cell was large and polyhedral (fig. 9). The cytoplasm was basophilic on polychrome stain and displayed a violaceous hue with eosin. Specific chromophilic granulation was usually absent, although occasionally faint red granules could be seen on Giemsa stain. The nucleus was eccentrically located, was of irregular size and shape, and might be round, oval, reniform, horseshoe-shaped,

lobulated, or folded upon itself. The chromatin was not densely packed and the nuclear membrane was sharply defined. At times multiple nucleoli were noted. The chromatin formed a lacy pattern composed of very fine intertwining strands with some thickening at the nodal points. Mitotic figures could be observed with moderate frequency. The size, shape, and appearance of the nucleus, and the tinctorial reaction of the cytoplasm, especially in Giemsa and oxydase stains, differentiated these cells from those of the granulocytic series.

*Lungs.* The lungs showed good aeration. Nodules composed largely of mononuclear cells were distributed throughout the parenchyma.



FIG. 6. PHOTOMICROGRAPH SHOWING THE PROLIFERATION OF MONOCYTIC CELLS IN THE INTERSTITIAL AND ADVENTITIAL TISSUES OF THE HEART ( $\times 160$ )

*Heart.* The heart exhibited considerable infiltration by large monocytic cells which were diffusely distributed throughout the epicardial surfaces, fat, and myocardium (fig. 6).

*Spleen.* There was marked disturbance of the architecture. The malpighian bodies were small and sparsely distributed throughout the parenchyma. They were composed, in the main, of both lymphocytes and reticulum cells distributed irregularly throughout the nodule. In the center of the follicle there was a striking deposition of acidophilic, albuminous material which formed clumpy masses between the cells. With reticulum stain, there was no notable increase in the argyrophilic fibrils. The capillaries were dilated and engorged; the endothelial cells were swollen and were desquamating into the lumen of the sinusoids. There was striking proliferation of the entire reticulo-endothelial system; the cells were very pleomorphic,

many multinucleated giant cells were present, and phagocytosis of iron, as shown by the Prussian blue reaction, was prominent. There was mobilization of the fixed reticulum cell elements and transformation, into free monocytoïd cells which were similar to the monocytic cells found in the peripheral blood. Mitotic figures were numerous. Fresh smears of the surface of splenic pulp demonstrated many large bizarre-shaped reticulo-endothelial cells in a state of hyperplasia and displaying erythrophagocytosis. These cells were closely related to the large peripheral blood monocytes.

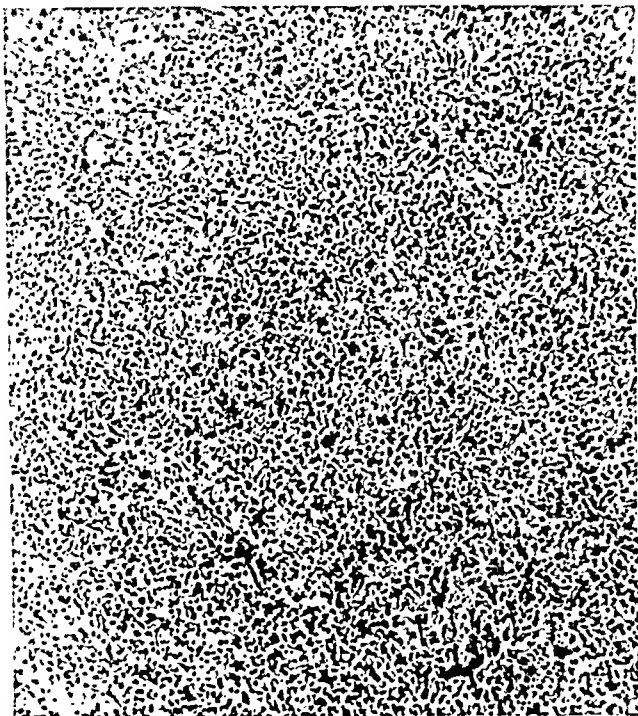


FIG. 7. PHOTOMICROGRAPH SHOWING THE DIFFUSE ACCUMULATION OF MONOCYTIC CELLS IN THE SINUSOIDS OF THE LIVER ASSOCIATED WITH ATROPHY AND NECROSIS OF THE LIVER CORDS ( $\times 200$ )

*Liver* (fig. 7). There was marked necrosis of the liver cells. The cords were shrunk and compressed. The Kupffer cells were markedly swollen and were choked with pigment, usually hemosiderin. The sinusoids were packed with freely circulating mononuclear cells, but the portal triads possessed large accumulations of fixed elements. Cells of the myelopoietic series were entirely absent.

*Splenic Pedicle.* Sections through the localized tumor of the tail of the pancreas, in the region of the ligated pedicle of the spleen, demonstrated large areas of foreign body reaction to the suture material. This proliferating granulation tissue was characterized by the presence of many engorged capillaries, pigment-containing macrophages, and multinucleated foreign body giant cells. Very noteworthy, how-

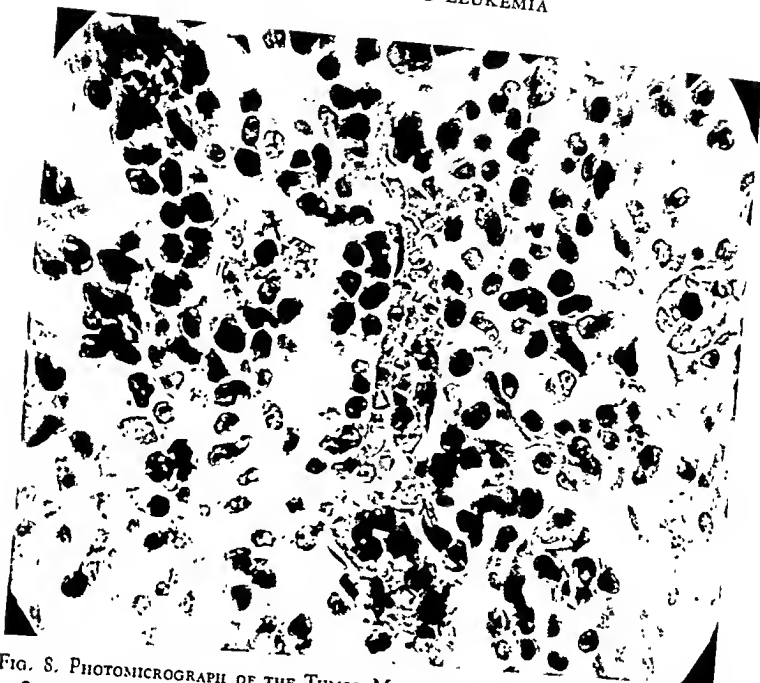


FIG. 8. PHOTOMICROGRAPH OF THE TUMOR MASS IN THE POSTOPERATIVE SPLENIC PEDICLE, SHOWING THE UNIFORM PROLIFERATION OF THE ADVENTITIAL AND RETICULAR CONNECTIVE TISSUES WITH DIFFERENTIATION INTO MONOCYTES. The paucity of granulocytes and fibroblasts is notable. ( $\times 360$ ).

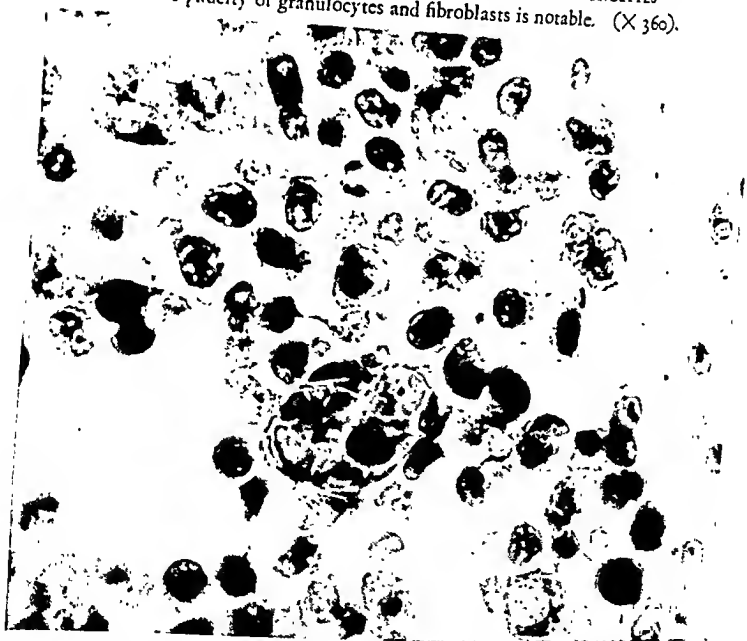


FIG. 9. HIGH POWER PHOTOMICROGRAPH OF TUMOR OF POSTOPERATIVE SPLENIC BED, SHOWING THE CHARACTERISTIC MONOCYTIC PROLIFERATION.

Note that the cells are not derived from peripheral blood by migration through the capillaries, but are products of the local connective tissue. ( $\times 500$ ).

ever, is the fact that the predominating cell was the large monocytic cell which has already been described in other organs (fig. 8), and many of which demonstrated active phagocytosis and a greater degree of pleomorphism than heretofore observed. It can be clearly demonstrated that the interstitial accumulation of these cells was not the result of migration of blood monocytes through the capillary walls but that it represented active adventitial proliferation (fig. 9). This admixture of monoblastic elements with other derivatives of mesenchyma represents clear-cut evidence of the ability of reticular connective tissue stem cells to differentiate into monocytes, thus supporting the previously expressed theory that

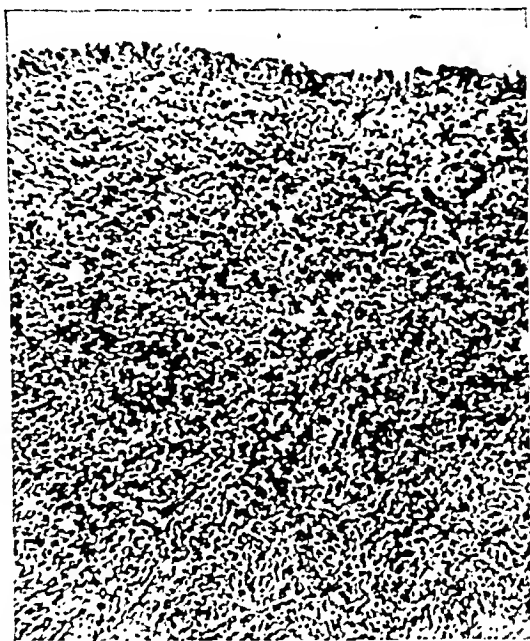


FIG. 10. PHOTOMICROGRAPH OF THE MASS IN THE RENAL HILUS, SHOWING THE BROAD SHEETS OF UNIFORM MONOCYTIC CELLS WHICH HAVE LARGELY REPLACED THE SUBMUCOSAL COATS OF THE RENAL PELVIS ( $\times 200$ )

monocytes are derived from mesenchymal tissue. Granulocytes, eosinophiles, and lymphocytes were almost entirely absent.

The brain, adrenal, and pituitary glands were not abnormal.

*Lymph Nodes (Para-aortic and Mesenterial).* The lymph nodes disclosed almost complete obliteration of the follicular structure. The bulk of the parenchyma was replaced by large, loosely packed sheets of monocytic cells which resemble those seen in the peripheral blood and other organs. The littoral and the reticulum cells showed marked numerical increase. The few vestiges of germinal centers were composed of swollen reticulum cells surrounded by deposits of fibrinous exudate. Phagocytosis of red cells, pigment, and nuclear remnants could be observed.

*Kidneys.* There was a diffuse and focal monocytic infiltration in the interstitium



FIG. 11. PHOTOMICROGRAPH DEMONSTRATING THE BROAD SHEETS OF UNIFORM CELLS FILLING THE BONE MARROW SPACES ( $\times 75$ )

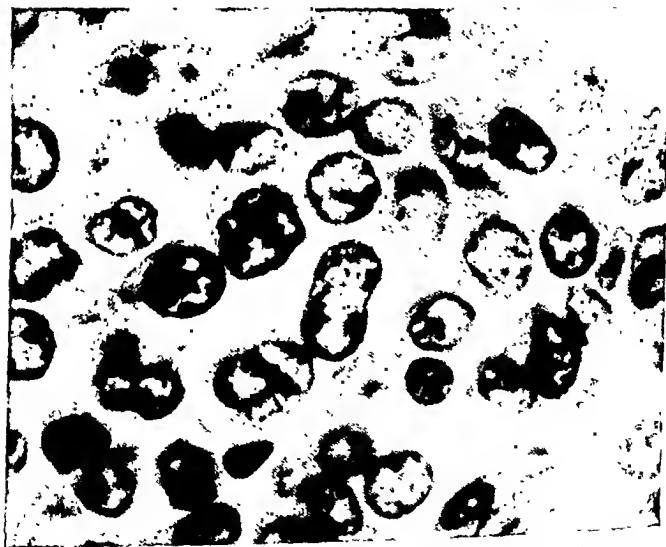


FIG. 12. PHOTOMICROGRAPHS OF BONE MARROW, SHOWING THE UNIFORM MONOCYTIC PROLIFERATION

There is almost complete absence of myeloid and erythroid elements, while only occasional normoblasts are seen. ( $\times 1515$ .)

of the adventitial tissues in the mid-portion of the organ. The subpelvic nodule and the large node in the renal pelvis were composed of monocytic cells similar to those already described (fig. 10).

*Bone Marrow.* Sections of medullary tissue of sternum, vertebra, and tibia displayed solid sheets of mononuclear cells which exhibited, in very typical manner, the morphologic characteristics concerned in this disease (figs. 11, 12). Cells undergoing mitosis could be found in abundance. Plasma cells and megakaryocytes were extremely rare. Except for an occasional eosinophilic myelocyte or granulocyte, there was almost complete absence of granulopoietic and erythrocytic precursors. A few monocytic cells possessed very faint, red granulations on Giemsa stain. This phenomenon may represent stem cell proliferation with slight differentiation toward the myeloid series. Oxydase stains of lymph node, liver, spleen, and bone marrow were completely negative.

*Thymus.* The normal architecture of the gland was altered by the presence of large sheets of pleomorphic monocytes which replaced the normally present thymocytes and medullary cells.

*Pituitary.* The pericapsular tissues and the anterior lobe were infiltrated by monocytic cells while none were seen in the posterior lobe.

#### DISCUSSION

The foregoing clinical and laboratory observations clearly indicate the erroneous conclusions which might have been drawn from single observations limited to a particular phase of this disease. Thus, at various times the features of agranulocytosis, aleukemic myeloid leukemia, aplastic anemia, leukemoid monocytosis, leukopenic infectious monocytosis, chronic splenic panhematopenia, and reticulo-endotheliosis were depicted. Since monocytic leukemia can pursue such an erratic course, it is not surprising that attempts to confine this syndrome within rigid limits have been unsuccessful.

Certain clinical features merit comment. The frequency and significance of hyperplastic gingivitis in this disease have been the subject of debate. A well marked gingivitis was recurrently present in this case in the earlier phases of the disease. Why, however, did this feature disappear when the full-blown manifestations of monocytic leukemia became apparent? It is clear that the statistical presence or absence of this sign depends somewhat on the period of observation. The striking clinical and hematologic remission is also noteworthy.

Granulocytopenia as an expression of occult leukemia is well recognized. Nevertheless, the clinical picture here was initially that of "idiopathic" agranulocytosis. The abrupt febrile onset, the rapid appearance of necrotizing and ulcerative lesions, and the prompt response to penicillin therapy favored this impression.

Kugel and Schnitker<sup>26</sup> have reported the value of penicillin in agranulocytosis elsewhere. This case was unique in affording controlled studies of the therapeutic effectiveness of penicillin compared with the usual agents employed in this disease. Penicillin therapy, instituted after the development of severe oral and perianal inflammation, resulted in prompt and dramatic improvement despite continuing granulocytopenia. Subsequent episodes of agranulocytosis were treated by penicillin alone; neither mucosal ulceration nor sepsis was detected clinically or on postmortem examination. From these observations it was concluded that penicillin was effective in combating the bacterial invasion of mucous membranes which frequently occurs when there is marked diminution or absence of circulating granu-

locytes. A corollary observation is that the effectiveness of penicillin *in vivo* is independent of the granulocyte. Penicillin has no effect on leukemia *per se*, but the prolongation of life through control of sepsis permitted this case to pursue the full gamut of monocytic leukemia symptomatology.

Our case conforms with most of the previously reported instances of monocytic leukemia in that this was a relatively acute illness in a young male. A striking feature of this syndrome was the contrast between the paucity of clinical manifestations and the progressive severity of the disease. The extensive pancardiac involvement was not detected clinically although an electrocardiogram taken shortly before death revealed nodal tachycardia and low voltage of QRS and T waves. Changes frequently noted in leukemia were either absent or unimpressive. Lymphadenopathy, splenomegaly, and hepatomegaly were mild; purpura and leukemic infiltrations of the skin were absent. Sternal pain and bony tenderness were present terminally only, at which time the liver had enlarged to 2-3 finger-breadths.

Similarly to the granulocytopenia, the relationship of the anemia to the leukemic process was somewhat obscure. Whereas the anemia was most pronounced in the early stage of the disease, this degree of anemia was not approached in the terminal stage. Some factor other than mechanical displacement of erythroid tissue must be logically assumed to explain not only the early profound reversible anemia but also the striking erythropoiesis during the remission period. It was also observed that large numbers of nucleated red blood cells appeared in the peripheral circulation at a time when samples of the bone marrow showed almost complete effacement of erythropoiesis. Generally there were persistent attempts at regeneration of red blood cells as manifested by the presence of macrocytosis and polychromasia.

As previously noted, there are a number of conflicting interpretations of the myeloid component of monocytic leukemia. Support for several of these divergent opinions could have been obtained from our case depending on the moment of observation. Thus, examination of the peripheral blood at times suggested a Nageli type due to the increase of myelocytes compared to monocytes. Myelocytes were particularly numerous in the granulocytopenic episodes and even more numerous in the recovery episodes. It is significant that in the prolonged preremission period following the first 3 episodes of granulocytopenia, the peripheral blood assumed a strong leukemoid character that went hand in hand with the restoration of a normal hematologic status. On the other hand, in the later stages of the disease, as the frank monocytic leukemia progressed, this myeloid reaction diminished markedly. We believe that this myeloid reaction was, for the most part, an expression of the bone marrow reactivity to the agranulocytosis and was not an expression of the monocytic leukemia. In part, too, the appearance of myeloid elements in the blood may be an irritation phenomenon secondary to monocytic infiltration of the bone marrow as suggested by Campbell, Henderson, and Croom.<sup>1</sup> The contention of Baserga,<sup>2</sup> previously quoted, is certainly pertinent here.

Supravital and Romanowsky stains proved adequate for the identification of the monocytic cells in the peripheral blood. In addition to the morphologic characteristics of these cells, the simultaneous appearance of monocytes, promonocytes, and monoblasts in the bone marrow further the identification of the "blast" cell,

one of the main stumbling blocks in the establishment of monocytic leukemia as an independent entity. This association of mature and immature forms in the bone marrow deserves emphasis. Because of the sporadic appearance in the blood of bizarre-shaped, forked and tailed mononuclear cells, one might have interpreted their presence as representing a benign reticulo-endotheliosis rather than monocytic leukemia. But, again, this impression would have been based on momentary observation and could not be borne out by prolonged study.

The gross findings at autopsy were generally unimpressive. As usual in monocytic leukemia, the involvement of the liver and lymph nodes was relatively mild and did not approach the degree usually found in either myeloid or lymphatic leukemia. Rather unusual, however, was the extensive leukemic infiltrations of the heart and thymus. The diffuse leukemic alterations in the omentum and the renal pelvic tissues are noteworthy, and the monocytic tumor formation at the site of ligature of the splenic pedicle is believed to be particularly significant. These monocytes are believed to have been derived from the local mesenchymal, adventitial, or reticular connective tissues. Their presence is therefore an indication of the retention of monocytopoietic potency by this tissue and may be looked upon as an expression of mesenchymal proliferation, a belief that has been advanced by Doan and Wiseman,<sup>14</sup> Jaffé,<sup>24</sup> Hadfield and Garrod,<sup>20</sup> and others. The site of origin of the monocyte should not be limited specifically to the RES, as many writers have proposed, but should be included in the broader concept of mesenchymal proliferation and differentiation. This concept would aid in resolving many of the conflicting opinions concerning the origin of the monocyte.

From all of the foregoing observations, it appears that the distinction between the Schilling and Naegeli types of monocytic leukemia is more apparent than real and does not deserve the rigid segregation frequently employed. A similar contention has been advanced by Campbell, Henderson, and Croom<sup>5</sup> and Custer.<sup>9</sup>

#### SUMMARY

Monocytic leukemia is an entity that has engendered a variety of divergent clinical and histologic opinions. A case is presented in which prolonged and intensive clinical and laboratory observations demonstrate the erratic course of this disease and illustrate the erroneous conclusions that may be derived from inconsistent, momentary observations of this dynamic process. Study of the "natural history" of monocytic leukemia yields observations which tend to reconcile many of the hitherto conflicting opinions regarding this disease.

The origin and characteristics of the monocytic cell are discussed, and its probable derivation from mesenchyma is emphasized.

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## ELECTROCARDIOGRAPHIC FINDINGS IN LEUKEMIA

By SAMUEL F. ARONSON, M.D., AND ELIE LEROY, M.D.

**A**LTHOUGH leukemic infiltrations of the heart are often encountered, particularly in the stem cell and myelogenous types of leukemia,<sup>1</sup> little attention in the literature has been given to their clinical recognition. Cardiac manifestations, almost always first discovered at necropsy, have been considered to be a part of the protean nature of the leukemic process and not a complication of the disease itself. However, occasionally symptoms produced by myocardial infiltrates have been the first or the most outstanding findings early in the course of the disease. Wintrobe and Mitchell,<sup>2</sup> in discussing atypical manifestations of leukemia, reported 2 such instances. One patient was treated for "heart disease" due to paroxysmal tachycardia and precordial discomfort for several months before myeloid leukemia was diagnosed. The other was a patient in whom the diagnosis of gallbladder and coronary diseases was made, and only at the autopsy was it found that the patient had a myeloid chloroma with widespread infiltrations. A 15 year old girl with a massive pericardial effusion as the most outstanding manifestation was later found to have a lymphatic leukemia with infiltration into the myocardium and pericardium.<sup>3</sup> Willis and Amberg<sup>4</sup> reported leukemic infiltration of the myocardium in a 2½ year old boy, in whom the main symptom of dyspnea could not be accounted for solely by the moderate secondary anemia which he manifested.

In reviewing 123 fatal instances of leukemia (0.86 per cent of 14,400 consecutive autopsies), Kirshbaum and Preuss<sup>1</sup> found that leukemic infiltration of the heart occurred in 43 of the cases (34 per cent). In 7 of these, the wrong clinical diagnosis of either rheumatic or arteriosclerotic heart disease had been made. An interesting case report of a patient with myelogenous leukemia was that of Blotner and Sosman. Their patient had a 2:1 A-V block which was treated with x-ray therapy applied directly to the heart area causing temporary disappearance of the block.<sup>1</sup>

The multiplicity of symptoms displayed by leukemic involvement of the heart is determined by the location and extent of the infiltrations and the commonly associated myocardial hemorrhages. So, one may note various disturbances of the cardiac rhythm<sup>5,6</sup> or symptoms of congestive heart failure. Since anemia may also cause weakness, palpitation, and dyspnea, since tachycardia may occur with the elevated basal metabolism often associated with leukemia, and since ascites may be due to either enlargement of the spleen or peritoneal involvement, these symptoms by themselves are not sufficient to make the diagnosis of cardiac infiltrations.

Early recognition of involvement of the heart, due to either leukemic infiltrations or to multiple small myocardial hemorrhages, with the prompt exhibition of the therapeutic measures commonly used in the treatment of heart failure, may

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afford the patient some relief from troublesome symptoms. Therefore, a review was undertaken to ascertain if the electrocardiogram might be of aid in making a diagnosis of leukemic involvement of the heart.

The following is a correlation of the electrocardiograms, autopsy findings, and clinical course of 8 patients with leukemia. Only pertinent data will be presented. In 5 patients, electrocardiograms were taken less than one month before death, and in the 3 others electrocardiograms were taken five weeks, two months, and seven months respectively before their death. Autopsy studies were made by one of us without knowledge of the histories or electrocardiographic interpretations. The electrocardiograms were interpreted before obtaining information of the clinical course or the autopsy results. Finally, the amassed material was examined for possible correlations.

#### CLINICAL DATA

The ages of these 8 patients ranged from 3 to 55 years. Four cases were diagnosed as lymphatic leukemia, 2 as blast or stem cell leukemia, and 2 as myelogenous leukemia. All of the patients had anemia with red blood cell counts varying between 80,000 and 3,500,000.

Five patients had clinical signs of either right or left heart failure. In all of these, physical examination revealed enlargement of one or more chambers of the heart. In the 3 instances where chest x-rays were made, the cardiac enlargements were confirmed. Three of the 5 patients had either systolic or diastolic murmurs or both.

Except for an apical systolic murmur in 1 instance, no clinical evidence of heart disease was present in the other 3 patients (see electrocardiograms, fig. 2 B, C, and D). Electrocardiograms of these 8 patients are shown in figs. 1 and 2.

#### ELECTROCARDIOGRAPHIC INTERPRETATIONS

Figure 1A, taken twenty-five days before death, shows a sinus tachycardia with an occasional ventricular premature systole (not shown in the record). S-T is depressed and T is small in all of the leads. T is diphasic in 1, 2, and the chest leads. QRS is upright in lead  $CF_2$ . The interpretation is: sinus tachycardia, ventricular premature systoles, probably combined heart strain, a definitely abnormal record.

Figure 1B, taken two weeks before death, shows a prolongation of the P-R interval to 0.24 second.  $R_1$  is tall and  $S_2$  and  $S_3$  are deep. S-T<sub>1</sub> is depressed and S-T<sub>3</sub> is elevated. T<sub>1</sub> is diphasic. QRS in lead  $CF_2$  is polyphasic. S-T is depressed in leads  $CF_4$  and  $CF_6$ . T is diphasic in lead  $CF_4$  and inverted in lead  $CF_6$ . The interpretation is: sinus rhythm, first degree A-V block, left heart strain, a definitely abnormal record.

Figure 1C, taken sixteen days before death, shows a tiny and diphasic QRS in lead 2. The T waves in leads  $CF_2$  and  $CF_4$  are inverted. The interpretation is: sinus rhythm, left axis shift, a definitely abnormal record.

Figure 1D, taken 20 days before death, shows a sinus tachycardia. QRS is small in lead 1; there is depression of the S-T segments, and the T waves are small in all of the limb leads. S-T is depressed in lead  $CF_4$  and T inverted in leads  $CF_2$  and  $CF_4$ . The interpretation is: sinus tachycardia, a definitely abnormal record.

Figure 2A, taken seven months before death, shows a QRS<sub>1</sub> which is-mainly

In summary, all of the electrocardiograms except 2B and 2D were interpreted as definitely abnormal records. The 2 exceptions were interpreted as borderline records. All the electrocardiograms taken on those patients with clinical evidence of heart disease were definitely abnormal. Contrariwise, the only 2 records which were not interpreted as definitely abnormal were taken on 2 of the 3 patients without clinical manifestations of cardiac involvement.

#### AUTOPSY FINDINGS

Postmortem examination was performed on all of the 8 patients. In 4 of them, leukemic infiltrates were noted in the various layers of the heart and particularly



FIG. 3. LEUKEMIC INFILTRATES IN THE MYOCARDIUM WITH DISRUPTION OF THE MUSCLE FIBERS (PATIENT WITH ELECTROCARDIOGRAM FIG. 1B). HEMATOXYLIN AND EOSIN STAIN.  $\times 100$

in the myocardium. These patients were among the 5 which presented signs of cardiac failure. (Fig. 3 shows a representative section from this group.)

In the 4 others, the small vessels of the myocardium were engorged with immature white cells. In 2 instances this capillary engorgement was associated with recent small foci of interstitial hemorrhages, and severe fatty degeneration was noted in another patient of this group. The latter had clinical evidence of left heart failure. (Fig. 4 shows a representative section from this group.)

No noteworthy arteriosclerotic changes were present in the coronary arteries of the 2 patients who were 55 years of age.

#### CORRELATION AMONG CLINICAL, ELECTROCARDIOGRAPHIC, AND AUTOPSY FINDINGS

The patients represented in electrocardiographic illustrations 1A, 1B, 1C, and 2A all had clinical evidence of heart failure with cardiac enlargement, and definitely

abnormal electrocardiograms. In these patients, autopsy examination revealed myocardial infiltrates. Therefore this group shows a close correlation between all methods of examination.

A fifth patient (electrocardiographic illustration 1D) had a large globular heart with "tigering" and fragmentation of myocardial fibers. Engorgement of the capillaries was noted, but no leukemic infiltrations of the heart layers were found. The electrocardiographic changes were nonspecific. The remaining 3 patients (electrocardiographic illustrations 2B, 2C, and 2D) had no clinical evidence of heart disease. In none of these were cardiac infiltrations noted, but the capillaries of the heart were engorged by leukemic cells in all instances. Again in this group of 4 patients, the clinical findings were confirmed by the autopsy evidence. The electro-

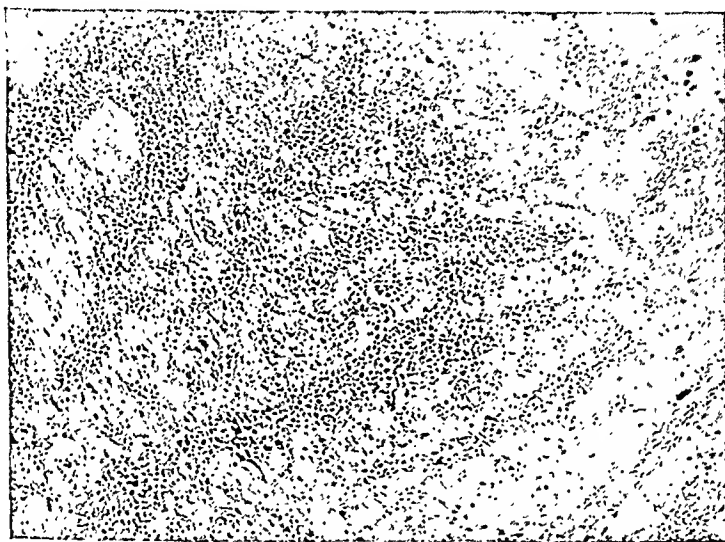


FIG. 4. DIFFUSE INTERSTITIAL HEMORRHAGE OF THE MYOCARDIUM WITH ENGORGEMENT OF THE SMALL BLOOD VESSELS (PATIENT WITH ELECTROCARDIOGRAM FIG. 2C).  
HEMATOXYLIN AND EOSIN STAIN.  $\times 100$

cardiographic abnormalities could be explained by the marked anemia and possible superimposed myocardial ischemia due to occlusion of the capillaries of the heart by leukemia cells.

The changes in the electrocardiogram depend upon the extent and location of the myocardial infiltrates. Similar electrocardiographic aberrations may occur, owing to unrelated conditions such as arteriosclerotic heart disease and transitory coronary insufficiency in older patients. Whenever the electrocardiogram is abnormal in a patient with leukemia, a careful search should be made for clinical evidence of heart disease. At times, the electrocardiogram may be the first evidence of myocardial infiltrates. It should be recalled that infiltrations of the heart are common in leukemia (34 per cent in one large series of autopsied cases<sup>1</sup>) and many varieties of heart disease may be simulated.

## CONCLUSIONS

1. The heart is frequently involved in leukemia.
2. There is a close correlation between the presence of leukemic myocardial infiltration, signs of heart disease, and abnormalities of the electrocardiogram.
3. The electrocardiographic changes do not constitute a diagnostic pattern.

The authors are indebted to Drs. L. N. Katz and O. Saphir for their suggestions and guidance.

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# SERUM PROTEIN CHANGES IN MYELOGENOUS AND LYMPHOCYTIC LEUKEMIAS AND HODGKIN'S DISEASE

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## INTRODUCTION

THERE are many reports in the literature reviewing Hodgkin's disease and myelogenous and lymphocytic leukemia.<sup>1,2</sup> In most of these the histologic and hematologic pictures are dealt with in detail, as are the various clinical criteria necessary for diagnosis. Occasionally an incidental notation is made of the serum protein values. Some investigators in the course of presenting data related to their particular problem have recorded the amount of albumin and globulin found in cases which happened to have Hodgkin's disease, myelogenous or lymphatic leukemia.<sup>3,4</sup> Another group of reports by Bing<sup>5,6</sup> and Bing and Plum<sup>7</sup> have stressed the finding of increased plasma and endothelial cells in the marrow or peripheral blood of patients having an increased amount of serum globulin.

Generally from these various sources one gains the impression that there are marked changes in plasma proteins in the aforementioned diseases, and that these may be related to the hematologic picture.

In order to ascertain the changes in the serum proteins in these diseases, a study was undertaken using analytical methods which have proved to be reliable. In particular, the use of methyl alcohol fractionation of albumins and globulins is known to give more accurate values than the usual salting-out technics.<sup>8</sup> In the present study serum protein determinations were carried out on a series of cases of Hodgkin's disease and myelogenous and lymphatic leukemias, and these were compared with a group of normals.

## METHODS

Twenty ml. of blood were drawn from patients and from normal subjects, using a clean dry syringe. Five ml. were placed in a bottle containing dry sodium oxalate and gently agitated to insure complete mixing of the anticoagulant. This sample was used for determining the sedimentation rate, according to the Wintrobe-Landsberg method,<sup>9</sup> and the hematocrit. The remainder of the original sample was delivered into a 15 ml. centrifuge tube and allowed to clot at room temperature. After retraction of the clot the tube was centrifuged at 3000 R.P.M. for 10 minutes and the supernatant serum transferred by means of a capillary pipet into a small test tube which was stoppered and refrigerated at 1° C.

Fractionation was done within 36 hours according to the method of Pillemer and Hutchinson.<sup>8</sup> This was carried out in a 1° C. cold room. The tube and contents to be fractionated were immersed in chipped ice and stirred mechanically while the methanol was slowly added from a buret. The globulin precipitate was removed by filtering through No. 42 Whatman paper. The samples containing the total protein

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and the albumin fraction were then stored in the cold until the analyses were to be carried out, which usually was from 1 to 10 days later. Marrow or peripheral blood studies made at the time of the collection of the sample were recorded.

The determination of total protein and albumin was done, using essentially the biuret method of Robinson and Hogden.<sup>10</sup>

*Preparation of Standard Curve.* Blood was collected from a normal subject in the manner previously described. A sample was set apart for a nonprotein nitrogen determination. One ml. was placed in a 10 ml. volumetric flask, which was made up to volume with 0.9 per cent sodium chloride solution. Three different aliquots in duplicate were pipetted in Kjeldahl flasks and total nitrogen determined. The factor 6.25 was used for converting nitrogen to protein. Duplicate aliquots of the same protein solution were pipetted into 15 ml. conical graduated centrifuge tubes. To each of these, saline was added to the 5 ml. mark and then 10 per cent trichloroacetic acid until the total quantity in each tube was 10 ml. These 6 tubes were centrifuged 15 minutes at 3000 R.P.M., or until the supernatant fluid was entirely clear. The liquid was then poured off and the tubes were inverted in a rack and allowed to drain for 5 minutes on filter paper. After this they were placed upright and 3 per cent sodium hydroxide solution added dropwise while stirring with a glass rod until solution was complete. Sodium hydroxide solution was then added to about 8 ml., and 0.25 ml. of 20 per cent copper sulfate solution was added to each tube. When this was well stirred the rods were washed off with more sodium hydroxide solution and the volume finally made up to 10 ml. The tubes were stoppered and shaken vigorously for one minute and allowed to stand for one-half hour, after which they were again centrifuged for 15 minutes at 3000 R.P.M. The supernatant fluid was transferred to a 19 mm. cuvet, and with distilled water as a blank, transmittances were read at 560 millimicrons on a Coleman No. 6 Junior spectrophotometer. A standard curve was prepared using semilogarithmic paper with transmittance represented on the abscissa and concentration in grams of protein per 100 ml. on the ordinate as determined by Kjeldahl analysis and corrected for nonprotein nitrogen. A straight line curve was obtained. For determination of total protein, 1 ml. of a 1:10 saline dilution of the sample was used. For the albumin determination, 0.5 ml. of a methyl alcohol supernatant was employed. Globulin was determined by difference.

*Classification of Disease.* The stages of the different diseases were classified as being early, moderately advanced, and advanced on the basis of available clinical, laboratory, and subjective information. Each case listed in the tables includes such an evaluation of clinical status at the time of blood sampling for serum protein determination.

*Results.* It is apparent from a comparison of the mean values of the three pathologic groups (tables 2, 3, 4) with the mean values of the normal group (table 1) that while there is no statistically significant change in the total proteins of the pathologic groups, there is a statistically significant decrease in albumin and an increase in globulin in the groups of Hodgkin's disease and myelogenous leukemia. No statistically significant change is seen to occur in the group of lymphocytic leukemias. This latter group, however, represents a rather small series and thus does

not exclude the possibility that the apparent trend toward a change from normal in the same direction as the other two groups might not be significant with a larger series.

While the magnitude of the changes from normal of the pathologic groups is small it is apparent from an examination of the data in tables 2 and 3 that 23 per cent of the Hodgkin's cases and 33 per cent of the myelogenous leukemia cases exceed the highest globulin value found in the normal group. Thus since the

TABLE 1.—*Serum Protein Levels in Normal Adults*

No.	Sex	Age	Albumin	Globulin	Total
1	M	30	4.92	1.36	6.28
2	M	32	4.78	1.88	6.66
3	M	24	4.82	2.25	7.07
4	M	22	4.72	2.84	6.56
5	M	31	5.34	2.14	7.48
6	M	26	4.82	2.36	7.18
5	F	20	4.50	1.80	6.30
8	F	21	4.40	2.22	6.62
9	F	20	4.64	1.58	6.22
10	F	22	4.08	2.44	6.52
11	F	23	4.40	1.42	5.82
12	F	20	4.60	2.58	7.18
13	F	22	4.12	2.10	6.22
14	M	20	4.92	2.16	7.08
15	M	19	4.48	2.36	6.84
16	M	22	5.40	1.96	7.36
17	F	46	5.12	1.64	6.76
18	M	32	4.60	1.90	6.50
19	M	61	4.13	1.97	6.10
20	M	23	4.08	2.48	6.56
21	M	22	4.69	1.87	6.56
22	M	30	4.72	2.10	6.82
23	F	29	4.69	2.08	6.77
Mean.....			4.65	2.07	6.72
Standard deviation....			0.36	0.34	0.41

majority of cases fall within the limits of the upper range of normal, the determination of serum globulin in cases of suspected Hodgkin's disease or myelogenous leukemia is of limited value. It should be noted that in the myelogenous leukemia group 12 of the 15 cases and in the Hodgkin's group 15 of the 26 cases had albumin levels below the lowest value in the normal group. In the lymphocytic leukemia group 4 of the 12 cases had albumin levels below the lowest normal value, and 2 of the 12 cases had globulin levels exceeding the highest normal globulin value.

Where individual cases had repeated analyses at different time intervals, there was a trend, in general, toward a decrease in total protein, usually with a larger decrease in albumin and a smaller increase in globulin. This trend appeared to be

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TABLE 2.—Serum Protein Levels in Patients with Hodgkin's Disease

No.	Sex	Age	Duration of illness from history (months)	Albumin	Globulin	Total	Clinical Status
1	F	32	108	4.36	4.59	8.95	Advanced
2	F	46	114	3.90	3.08	6.98	Advanced
3	M	42	84	4.40	2.68	7.08	Advanced
4	M	30	90	3.38	2.30	5.68	Advanced
5	F	29	45	5.35	1.98	7.33	Mod. adv.
6	F	41	48	4.54	1.99	6.53	Mod. adv.
7	M	70	54	3.81	3.99	7.80	Mod. adv.
8	F	44	252	2.94	3.62	6.56	Mod. adv.
9	F	60	257	3.99	2.81	6.80	Mod. adv.
10	M	52	27	3.38	2.44	5.82	Mod. adv.
11	M	62	48	4.36	3.26	7.62	Mod. adv.
12	M	27	51	3.45	2.08	5.53	Advanced
13	F	23	27	2.80	2.80	4.60	Advanced
14	M	24	24	2.54	3.80	6.40	Advanced
15	F	19	28	3.72	3.54	7.26	Mod. adv.
16	M	28	36	2.94	3.38	6.32	Mod. adv.
17	F	23	41	5.08	1.93	7.01	Mod. adv.
18	F	51	9	4.13	1.39	5.52	Mod. adv.
19	M	39	24	3.90	1.54	5.44	Mod. adv.
20	F	35	108	3.48	2.43	5.91	Advanced
21	M	38	114	4.42	2.05	6.47	Mod. adv.
22	F	42	6	1.82	1.82	6.24	Mod. adv.
23	M	49	6	4.78	1.78	6.56	Early
24	F	39	8	4.56	1.72	6.28	Early
25	M	49	36	5.18	2.03	7.21	Early
26	F	51	42	3.27	2.11	5.38	Mod. adv.
27	M	39	6	3.80	2.12	5.92	Advanced
28	F	35	84	3.86	1.66	5.52	Advanced
29	M	44	144	4.42	1.86	6.28	Mod. adv.
30	F	42	150	4.63	2.69	7.32	Mod. adv.
31	M	59	42	3.95	2.51	6.56	Mod. adv.
32	F	43	108	4.11	2.19	6.30	Mod. adv.
33	M	39	110	4.56	2.00	6.56	Advanced
34	F	49	9	3.60	3.04	6.64	Advanced
35	M	49	49	2.76	2.96	5.72	Early
36	F	44	60	3.98	3.83	7.81	Mod. adv.
37	M	38	36	4.42	3.12	7.54	Mod. adv.
38	F	44	60	5.25	1.82	7.07	Mod. adv.
39	M	38	36	4.42	2.58	7.00	Mod. adv.
Mean.....			4.07	2.49	6.56		
Standard deviation....			0.68	0.68	0.70		
Probability of chance difference* .....			0.00	0.00	0.32		

\* Probability of differences due to chance was obtained by means of the critical ratio and a table of probabilities.

$$\text{critical ratio} = \frac{\text{difference between means}}{\text{standard error of difference}}$$

A value of zero indicates that differences from normal are not due to chance, while a value of 0.20, for example, indicates that chance variation from normal may occur in 2 out of 10 experiments.

particularly apparent as the disease advanced and approached fatal termination (see cases 2 and 15, table 3). In some instances of early disease there appeared to be some improvement in the albumin and globulin levels toward normal following x-ray therapy. Estimation of the size of the liver and spleen by palpation failed to

TABLE 3.—Serum Protein Levels in Patients with Myelogenous Leukemia

No.	Sex	Age	Duration of illness from history (months)	Albumin	Globulin	Total	Clinical Status
1	M	41	2	3.36	3.35	6.71	Acute terminal
2	M	53	6	5.20	3.33	8.53	Early
			9	3.72	3.54	7.26	Early
			11	2.94	3.65	6.59	Mod. adv.
3	M	70	60	3.90	3.36	7.26	Mod. adv.
4	M	52	54	4.36	2.90	7.26	Mod. adv.
			59	3.90	2.09	5.94	Mod. adv.
5	F	48	48	4.17	1.55	5.62	Mod. adv.
			51	3.66	2.74	6.40	Mod. adv.
6	F	58	48	4.73	2.71	7.44	Mod. adv.
			51	3.80	2.60	6.40	Mod. adv.
			53	3.95	2.53	6.48	Mod. adv.
7	F	25	30	4.96	1.84	6.80	Mod. adv.
			33	4.40	2.16	6.56	Mod. adv.
8	M	40	24	3.64	2.26	5.90	Mod. adv.
9	M	74	48	3.90	2.84	6.74	Advanced
10	F	80	1	3.56	3.84	6.40	Mod. adv.
11	M	35	48	3.46	2.06	5.52	Advanced terminal
12	M	71	1	2.76	2.18	4.94	Advanced (aleukemic)
13	M	30	24	4.33	2.02	6.36	Mod. adv.
14	M	35	6	4.18	2.72	6.90	Mod. adv.
15	M	72	9	3.63	2.09	5.72	Mod. adv. (aleukemic)
			12	3.63	2.02	5.65	Mod. adv. (aleukemic)
			16	2.91	2.25	5.16	Advanced terminal leukemic phase
Mean.....				3.80	2.65	6.46	
Standard deviation....				0.46	0.61	0.72	
Probability of chance difference*....				0.00	0.00	0.20	

\* See footnote, table 2.

reveal any relationship between the size of these organs and the serum protein values. However, the over-all extent of enlargement of these organs was difficult to evaluate because of x-ray therapy directed toward reducing the size of these organs. In general, however, as the disease progressed these organs tended to become enlarged.

In view of the reports that serum globulin increases are associated with an in-

crease in plasma cells<sup>5-7</sup> and that the gamma globulin level of blood may be related to the lymphocytes,<sup>11</sup> careful differential counts on peripheral blood from all the different cases were carried out. There was no apparent relationship between the level of any particular cellular component of the peripheral blood and the level of either albumin or globulin. In no instance were plasma or endothelial cells found in the peripheral blood. Bone marrow smears made in about one half of the leukemia cases revealed the presence of plasma or endothelial cells in significantly high numbers only in cases 12 and 15 of the myelogenous leukemia group (table 3).

TABLE 4.—Serum Protein Levels in Patients with Lymphocytic Leukemia

No.	Sex	Age	Duration of illness from history (months)	Albumin	Globulin	Total	Clinical Status
1	M	51	24	4.36	1.84	6.20	Mod. adv.
2	M	46	18	4.36	3.86	8.20	Mod. adv.
3	F	56	63	5.08	1.67	6.75	Mod. adv.
4	M	55	54	5.08	1.61	6.71	Early
5	F	51	98	4.72	2.54	7.26	Mod. adv.
			102	3.98	1.54	5.52	Mod. adv.
			104	4.54	2.02	6.56	Mod. adv.
6	F	70	45	4.42	2.38	6.80	Mod. adv.
7	F	60	6	3.36	2.76	6.12	Mod. adv.
8	F	68	6	3.98	3.20	7.18	Mod. adv.
9	M	72	18	4.50	2.16	6.66	Mod. adv.
			20	4.04	2.24	6.28	Mod. adv.
10	M	44	108	4.20	1.78	5.98	Mod. adv.
11	F	56	3	3.99	2.07	6.05	Mod. adv. (leukemic)
12	F	50	12	4.78	2.22	7.00	Mod. adv. (leukemic)
Mean.....				4.38	2.27	6.65	
Standard deviation.....				0.48	0.68	0.63	
Probability of chance difference*...				0.20	0.34	0.74	

\* See footnote, table 2.

The marrow smear from case 12 had 1 per cent plasma cells and 1.2 per cent unclassified cells which resembled endothelial cells, while the smear from case 15 revealed the presence of 20.6 per cent unclassified cells suggestive of an endothelial origin. In both of these cases the serum globulin levels were below normal. Thus no relationship can be seen from this group of cases between the serum globulin level and the bone marrow cellular composition.

The levels of hemoglobin and erythrocytes were found to be unrelated to the serum protein level. However, a true evaluation was made difficult by the fact that most of these patients received generous and frequent transfusions of whole blood. In general, it can be said that as the terminal state was approached the hemoglobin and erythrocyte levels decreased along with the albumin and total protein values.

## COMMENT

That only one third of the cases of the myelogenous leukemia group and only one fourth of the Hodgkin's group showed increases in the serum globulin levels raises the question as to the significance of these changes in these conditions. The finding of a higher percentage of cases in both groups showing a decrease in albumin is consistent with the nutritional failure associated with chronic wasting disease. A point worth emphasizing is that in the cases of hyperglobulinemia the total protein levels were within normal limits, owing to the simultaneous decrease in serum albumin. The inadequacy of a total protein determination as a measure of serum protein change thus is obvious.

In multiple myeloma, a disease usually associated with a marked hyperglobulinemia, Gutman et al.<sup>12</sup> found that 63 per cent of 38 cases studied had elevated globulin levels. Not only did the multiple myeloma group show an incidence of hyperglobulinemia 2 to 2.5 times that reported here for myelogenous leukemia and Hodgkin's disease, but the levels of globulin in the multiple myeloma series with hyperglobulinemia were much higher than those reported in the present paper. Thus when compared to a disease like multiple myeloma, the globulin changes in myelogenous leukemia and Hodgkin's disease are not particularly striking.

It is important to recognize that the serum globulin fraction is not a single entity and that it is actually made up of a large number of different proteins. It is conceivable that significant changes in one or more of the globulin components may occur in cases of Hodgkin's disease and myelogenous and lymphocytic leukemia without being reflected in the total globulin determination. However, electrophoretic analyses of a similar series of cases will be necessary before such a possibility can be evaluated.

## SUMMARY

1. Using a methyl alcohol fractionation technic, the albumin, globulin, and total protein levels were determined in a series of normal adults and compared with cases of myelogenous and lymphocytic leukemias and Hodgkin's disease.
2. Statistically significant decreases in albumin and increases in globulin were found in the cases of Hodgkin's disease and myelogenous leukemia, but without significant changes in total protein. Globulin levels above the highest normal value were found in 23 per cent of the former and 33 per cent of the latter group.
3. No apparent relationship was noted between the levels of the serum protein fractions and (1) the hemoglobin level, (2) the erythrocyte count, (3) the peripheral white blood cell picture, and (4) the bone marrow smears.

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# THE IMPROVED DEMONSTRATION OF CIRCULATING ANTIBODIES IN HEMOLYTIC ANEMIA BY THE USE OF A BOVINE ALBUMIN MEDIUM

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THE pathogenetic mechanisms of the excessive hemolysis that occurs in both familial and acquired cases of hemolytic anemia continue to be the subject of much discussion and investigation. Several theories have been advanced to explain these processes, in which spherocytosis of the red cells commonly occurs. In 1938, Dameshek and Schwartz<sup>1</sup> first demonstrated that the spherocyte was not necessarily the result of an hereditary defect of erythropoiesis, but could be produced experimentally by means of an immune hemolytic serum. These studies indicated that a circulating hemolytic antibody might be the cause of the excessive hemolysis and of the spherocytosis found in many cases of acquired hemolytic anemia and possibly in congenital spherocytic hemolytic anemia as well. Further studies<sup>2</sup> demonstrated that various types of antibodies, both hemolysins and agglutinins, caused injury to red cells with resulting complete breakdown (hemolysis) or incomplete breakdown, i.e. spherocytosis.

By means of the customary technics for demonstrating hemolysins and agglutinins using salt solution as a diluent, these antibodies were found in only occasional cases of acquired hemolytic anemia, usually of the acute variety. The independent demonstration in 1944 by Race<sup>3</sup> in England and Wiener<sup>4</sup> in this country of a so-called "blocking" or "incomplete" type of Rh antibody demonstrable only in a serum medium raised the possibility that circulating antibodies of this type might also be detected in patients with hemolytic anemia. Diamond and Denton<sup>5</sup> in their investigations of Rh antibodies suggested the use of bovine albumin as a diluting medium, and we have used this method in our search for antibodies in cases of hemolytic anemia. By this method, abnormal iso-antibodies have been discovered in a number of patients with hemolytic anemia in whom negative or questionable results were obtained by the ordinary technics.

## METHODS AND MATERIAL

A tube agglutination method was used, duplicate systems being set up in normal saline and in 20 per cent bovine albumin solution in small lipless test tubes 7 x 70 mm. Search was made for auto- and iso-hemolysins and auto- and iso-agglutinins at 37° C., 22° C., and 3° C. Two sets of serial dilutions (1:2, 1:4, 1:8, etc.) of the serum to be tested were made, one in normal saline and one in bovine albumin solution. In testing for *autoagglutinins*, the patient's red cells were washed in normal saline, all the saline was removed, and the cells were then resuspended separately in saline and in bovine albumin solution to make an approximate 2

From the Blood Laboratory of the J. H. Pratt Diagnostic Hospital and Boston Dispensary and from the Department of Medicine, Tufts College Medical School. Aided by grants from the Charlton Fund and interested donors.

per cent suspension in each medium. Similar suspensions were made of a random group O blood for use in the tests for *iso-agglutinins*, two drops of the red cell suspension being mixed with two drops of the serum dilution with a suitable control. In the experiments for hemolysins, fresh guinea pig serum, diluted 1:10 in saline and in bovine albumin, was used for complement. For the demonstration of *auto-hemolysins*, two drops of the patient's cell suspension, one drop of complement 1:10, and two drops of the respective serum dilution were placed in each test tube. For *isohemolysins*, two drops of a group O red cell suspension, one drop of complement, and two drops of the serum dilution were used. A control of red cell suspension and complement alone was always included. When using the saline medium, a saline red cell suspension and saline dilutions of the serum were used; while with the determinations using bovine albumin no saline was allowed to enter the system, all dilutions being made with albumin. The tubes were mixed by shaking manually, then allowed to react at 37° C., 22° C., and 3° C. for at least two hours. The supernatant fluid was examined for hemolysis and the sediment then examined for agglutination both macroscopically, using a 6 X hand lens, and microscopically, when the reaction was doubtful or negative.

Five cases of acquired hemolytic anemia (idiopathic type), 3 patients with familial spherocytosis, 2 with severe familial Mediterranean anemia, 2 with sickle cell disease, and 1 patient with paroxysmal nocturnal hemoglobinuria were studied. In addition, 1 case of severe acute hemolytic anemia caused by chemical or drug exposure, 2 with severe "hypersplenic" pancytopenia with hemolytic anemia, and 1 with hemolytic anemia symptomatic of subacute lymphatic leukemia were studied (table 1). The primary diagnosis of a hemolytic disease was established in the customary manner by various clinical and laboratory studies, including routine blood counts, platelet counts, reticulocyte determinations, examinations of fresh and stained blood smears as well as by tests of the hypotonic fragility of the red cells, serum bilirubin levels, and fecal urobilinogen excretion. In several cases, parallel determinations of the survival time of transfused red cells were made (table 1).

#### RESULTS

In 4 of the 5 cases of acquired hemolytic anemia, a circulating warm antibody that could not be detected in a saline medium was repeatedly demonstrable in the bovine albumin system. In the other patient a warm agglutinin, weakly reactive in saline, was found to react in higher dilutions and to give more intense reactions in the bovine albumin medium (tables 1, 2, 3). The 3 patients with congenital spherocytic anemia gave interesting contrasting results. When afebrile and not in crisis, no circulating antibodies could be detected. Thus patient Philip M. at no time showed a circulating antibody. However, during a hemolytic crisis which occurred in one of these patients (Pauline O'N.) a warm autohemolysin in a titre of 1:32 was demonstrated at 37° C. in bovine albumin solution only (table 4). Repeated examinations of this patient's blood after the crisis and after splenectomy have consistently failed to disclose this antibody again. The third patient (Genevieve S.) ran an irregularly febrile course for the first two months

of observation interspersed with numerous "colds" and feverish "grippy" attacks. During these two months, an agglutinin weakly reactive in saline and somewhat more active in bovine albumin solution was present which agglutinated the patient's own cells and sporadic group A and group O cells at 37° C. Later when the patient became afebrile, this agglutinin could not be disclosed.\*

In the 2 patients with sickle cell anemia, the 2 patients with severe Mediterranean anemia, and the patient with paroxysmal nocturnal hemoglobinuria, antibodies were lacking in both saline and bovine albumin solution. Antibodies were also absent in the patient with severe acute hemolytic anemia caused by exposure to chemicals, in 2 patients with a hemolytic process associated with severe hypersplenic pancytopenia, and in another patient with symptomatic hemolytic anemia secondary to subacute lymphatic leukemia.

#### DISCUSSION

Demonstration of an iso-antibody in patients with hemolytic anemia may help to differentiate between acquired and congenital hemolytic anemia. This was first pointed out in 1908 by Widal, Abrami, and Brulé,<sup>6</sup> who emphasized the diagnostic importance of autoagglutinins as indicative of the acquired cases. Although Dameshek and Schwartz<sup>1</sup> later stressed the finding of hemolysins, it must be admitted that both hemolysins and agglutinins are not often detectable in a saline medium. From our present results, it seems probable that this difficulty can to a large extent be obviated by the use of bovine albumin solution as a diluting medium. With this method, a warm iso-antibody was disclosed in every instance of "idiopathic" acquired hemolytic anemia we have recently studied. Iso-antibodies were not demonstrated in other cases of acquired hemolytic anemia due to chemical, "hypersplenic," and other (i.e., "symptomatic") causes. Not only has the finding of an abnormal iso-antibody been of diagnostic value but it has also been of some prognostic aid in that complete assurance of permanent cure following splenectomy cannot be given to these patients.

Too sweeping conclusions cannot be deduced from the finding of a circulating hemolysin in the 1 patient with congenital spherocytic anemia during a hemolytic crisis. The diagnosis of congenital spherocytic anemia in this patient is well established by its presence in four generations of the family. Additional examinations of the serum by this method in patients with familial spherocytic anemia must be made during crisis before definite conclusions can be drawn. However, this finding, if substantiated by further positive determinations, suggests the possibility that in congenital spherocytic anemia there may be a circulating antibody of such low titre that it is demonstrable only during a period of crisis when increased amounts of the hemolytic antibody are present in the circulation. As a corollary, one might speculate further and visualize a crisis as the result of a sudden elevation of hemolytic antibody titre.

In several of our cases with circulating antibodies detected by the albumin technic, parallel determinations of the survival time of normal red cells introduced

\* We are grateful to Dr. Philip Levine for confirming these observations in his laboratory.

TABLE 1.—Summary of Pertinent Hematologic Findings in Seventeen Patients with Hemolytic Anemia

Diagnosis	Case No.	Patient	Age	Hgb., Gm./100 cc.	RBC Mill/mm. <sup>3</sup>	Spherocytosis	Reticulocytes %	Serum Bilirubin mg. %	Circulating Antibodies 37° C.		Remarks
									Saline	Albumin	
Acquired hemolytic anemia—idiopathic	1	Evelyn H.	9	5.6	2.38	Marked	22.5	2.8	0	1:32	Presplenectomy. Antibody persisted postsplenectomy 1:16. Transfused red cells survived 55 days, exponential curve.
	2	Frank S.	52	9.0	2.60	Marked	14.1	4.2	0	1:16	Postsplenectomy. Severe ulcerative colitis. Transfused red cells survived 42 days, exponential curve.
	3	Sonia B.	18	10.4	3.11	Slight	1.9	1.5	1:2	1:64	Postsplenectomy. Transfused red cells survived 42 days, exponential curve.
	4	Evelyn S.	19	8.7	3.32	0	24.8	2.8	0	1:8	Postsplenectomy. Transfusion every three weeks.
	5	Ellen L.	58	8.3	2.62	Marked	30.2	5.7	0	1:32	Postsplenectomy. Transfused red cells survived 30 days, exponential curve.
Congenital spherocytic anemia	6	Philip M.	7	8.7	3.31	Marked	16.9	1.8	0	0	Presplenectomy.
	7	Pauline O'N. (in crisis)	17	10.7	3.20	Marked	8.2	3.3	0	1:32	Presplenectomy. Transfused red cells survived 38 days, exponential curve.
		Pauline O'N. (not in crisis)		12.3	3.70	Slight	7.2	1.4	0	0	Postsplenectomy. Transfused red cells survived 103 days, straight line curve.
Acquired hemolytic anemia—chemical	8	Genevieve S.	35	12.1	3.55	Marked	8.0	2.0	1:1	1:2	Presplenectomy. Transfused red cells survived 99 days, straight line curve.
	9	James P. C.	18	9.5	2.60	0	9.2	1.0	0	0	Recovery with transfusions. Transfused red cells survived 95 days, straight line curve.

Paroxysmal nocturnal hemoglobinuria	10	Jessie H.	41	9.0	3.4	0	10.1	1.5	0	0	Transfusion every 2 weeks.
Mediterranean anemia (severe)	11	Ferdinand L.	13	6.9	3.35	0	9.1	2.8	0	0	Transfused red cells survived 92 days.
	12	Rosanne A.	1	9.8	3.30	0	2.0		0	0	Transfused red cells surviving normally after 85 days.
Sickle Cell anemia	13	David P.	11	8.5	2.40	0	10.8	4.0	0	0	Transfused red cells survived 114 days.
	14	Amos W.	26	11.7	3.89		4.9	1.0	0	0	
Symptomatic hemolytic anemia	15	Alex D.	49	5.4	1.60	Minimal	1.6	2.8	0	0	Subacute lymphatic leukemia. Transfusion every week.
Hypersplenic hemolytic anemia	16	Maurice H.	66	6.9	1.80	0	7.8	1.9	0	0	Presplenectomy. Improvement postsplenectomy.
	17	Catherine B.	53	5.8	1.74	Slight	8.1	2.0	0	0	Presplenectomy. Improvement postsplenectomy.

into the patient's circulation were performed using the Ashby technic.\* These showed a diminished survival time, indicating an action of abnormal hemolytic antibody against the introduced red cells. These results differed sharply from the

TABLE 2.—*Agglutinins in Saline and Albumin Media*  
Case 1—E. H.—Acquired Hemolytic Anemia—Presplenectomy  
Autoagglutinins: Serum E. H. vs. Cells E. H.

Temp.	Cells	Serum E. H.—Saline Dilutions								
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	Control
37° C.	E. H. Cells Saline suspension	—	—	—	—	—	—	—	—	—
25° C.		+	—	—	—	—	—	—	—	—
3° C.		+	—	—	—	—	—	—	—	—
Temp.	Cells	Serum E. H.—Albumin Dilutions								
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	Control
37° C.	E. H. Cells Albumin suspension	+++	++	++	++	++	+	—	—	—
25° C.		++	++	++	+	+	—	—	—	—
3° C.		++	++	+	—	—	—	—	—	—

Isoagglutinins: Serum E. H. vs. # 332 O Cells.

Temp.	Cells	Serum E. H.—Saline Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	# 332 O cells Saline suspension	—	—	—	—	—	—	—	—
25° C.		—	—	—	—	—	—	—	—
3° C.		++	+	—	—	—	—	—	—
Temp.	Cells	Serum E. H.—Albumin Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	# 332 O cells Albumin suspension	++	++	+	—	—	—	—	—
25° C.		++	+	—	—	—	—	—	—
3° C.		+++	++	++	—	—	—	—	—

cases in which antibody could not be detected. In addition it was possible to study the contrast between the survival time of introduced red cells in and out of crisis in the case of congenital spherocytosis which showed an autohemolysis during crisis. During crisis red cell destruction was rapid, whereas after splenec-

\* These studies will be reported upon in another communication.

TABLE 3.—*Hemolysins in Saline and Albumin Media*

Case 2—F. S.—Chronic Acquired Hemolytic Anemia—Postsplenectomy

Autohemolysins: Serum F. S. + Complement 1:10 vs. Cells F.S.

Temp.	Cells	Serum F. S.—Saline Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	F. S. Cells Saline suspension	—	—	—	—	—	—	—	—
25° C.		—	—	—	—	—	—	—	—
3° C.		—H +++A	—H +A	—	—	—	—	—	—
Temp.	Cells	Serum F. S.—Albumin Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	F. S. Cells Albumin suspension	+H +++A	++H +++A	+H +++A	~H ++A	—H ++A	—H	—	—
25° C.		++H +++A	+H +++A	—H ++A	~H +A	—	—	—	—
3° C.		—H +++A	—H +A	—H +A	—	—	—	—	—

Isohemolysins: Serum F.S. + Complement 1:10 vs. # 237 O Cells.

Temp.	Cells	Serum F. S.—Saline Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	# 237 O cells Saline suspension	—	—	—	—	—	—	—	—
25° C.		—	—	—	—	—	—	—	—
3° C.		—	—	—	—	—	—	—	—
Temp.	Cells	Serum F. S.—Albumin Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	# 237 O cells Albumin suspension	—H +++A	—H +A	—H +A	—	—	—	—	—
25° C.		—H +++A	—H +++A	—H +A	—	—	—	—	—
3° C.		—H +++A	—H +A	—	—	—	—	—	—

H = Hemolysis.

A = Agglutination.

omy a normal survival time was present. The contrast in the survival time of the red cells in the same patient during crisis and after splenectomy was very striking.

TABLE 4.—*Hemolysins in Saline and Albumin Media*

Case 7—P.O.N.—Familial Spherocytic Anemia—Crisis—Presplenectomy

Autohemolysins: Serum O.N. + Complement 1:10 vs. Cells O.N.

Temp.	Cells	Serum O.N.—Saline Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	O.N. Cells Saline suspension	—	—	—	—	—	—	—	—
25° C.		—	—	—	—	—	—	—	—
3° C.		—	—	—	—	—	—	—	—
Temp.	Cells	Serum O.N.—Albumin Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	O.N. Cells Albumin suspension	+++H	+++H	++H	++H	++H	÷H	—	—
25° C.		+++H	+++H	++H +A	++H +A	÷H +++A	—H +++A	—H ÷A	—
3° C.		—H +++A	—H +++A	—H +A	—	—	—	—	—

Isohemolysins: Serum O.N. + Complement 1:10 vs. #138 O Cells.

Temp.	Cells	Serum O.N.—Saline Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	#138 O cells Saline suspension	—	—	—	—	—	—	—	—
25° C.		—	—	—	—	—	—	—	—
3° C.		—	—	—	—	—	—	—	—
Temp.	Cells	Serum O.N.—Albumin Dilutions							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	Control
37° C.	#138 O cells Albumin suspension	++H	++H	++H	÷H	—	—	—	—
25° C.		—	—	—	—	—	—	—	—
3° C.		—H +++A	—H +++A	—H +A	—H —A	—	—	—	—

## Postsplenectomy

Repeat studies on June 28, August 26, and October 7, 1946, showed a cold agglutinin to a titer of 1:4 in albumin alone. No warm agglutinins or hemolysins were found.

H = Hemolysis.

A = Agglutination.

Various tests are now at hand for discriminating between familial and acquired hemolytic anemia. Since spherocytosis may be present in both, it is not diagnostic.

The results of splenectomy are often revealing, providing spherocytosis disappears, in which case acquired hemolytic anemia can be diagnosed. If spherocytosis is still present, the distinction may still be in doubt. By using (1) serum tests for abnormal antibodies, particularly with the bovine albumin technic, (2) tests of the red cells with antiglobulin serum (cf. below), and (3) survival time experiments, the discrimination between acquired cases due to auto-immunization and familial cases can almost always be made. This is often of diagnostic and prognostic importance. Furthermore, the complete lack of any type of antibody in cases of acquired hemolytic anemia points to a type of hemolysis which is due to some other mechanism such as chemical poisoning, "hypersplenism," and the like.

The finding of iso-antibodies by the use of bovine albumin rather than salt solution as a diluting medium raises some interesting questions as to the nature of the reaction and of the antibody. The use of serum and albumin as diluting media originated with investigation of the anti-Rh agglutinins which could not be detected in saline media. These were first described as "blocking"<sup>4</sup> antibodies; i.e., they were thought to "coat" or otherwise affect the red cell rendering it incapable ("blocking" it) of reacting further with anti-Rh agglutinin. Later, they were considered to be "univalent" or "incomplete"<sup>4,3</sup> antibodies because they required a serum constituent to effect agglutination, in contradistinction to "complete" or "bivalent" antibodies which reacted in simple salt solutions. Although these explanations are attractive, it is possible that neither is correct but that the serum or albumin solution is a more physiologic medium, allowing the demonstration of antibodies that cannot be detected with the less physiologic salt solution.

Dameshek and Schwartz in 1940<sup>7</sup> postulated that in certain cases of hemolytic anemia which failed to show hemolysin in the serum, the antibody might be adsorbed to the red blood cell. Coombs, Mourant, and Race<sup>8</sup> found this condition to be true of certain cases of acute hemolytic disease of the newborn (erythroblastosis foetalis) as determined by the use of an anti-human globulin rabbit serum.<sup>9</sup> This serum was produced by injecting human serum or serum globulin in rabbits and thus producing an antiglobulin (anti-antibody) material.<sup>9</sup>

By means of this serum, Coombs and his co-workers<sup>8</sup> and Hill and Haberman<sup>10</sup> obtained positive agglutination of the infant's red cells in cases of erythroblastosis foetalis when an anti-Rh agglutinin could not be found in the mother's serum. More recently, Boorman, Dodd, and Loutit<sup>11</sup> studied cases of acquired and congenital hemolytic anemia with anti-human globulin rabbit serum. Positive results, i.e. agglutination, were obtained in the 5 acquired cases, negative results in the 17 familial cases. These results indicated, according to these observers, that the red cells of acquired hemolytic anemia contained adsorbed agglutinin which was the cause of the hemolytic process.

#### SUMMARY

1. Bovine albumin solution as a testing and diluting medium was used for the detection of abnormal iso-antibodies in the serum of various cases of hemolytic anemia.

2. Five patients with idiopathic acquired hemolytic anemia all showed a warm

agglutinin, although in 4 of these patients antibody could not be detected by use of the standard saline technic. Higher titres than in salt solution were always obtained in the albumin medium. Three patients with congenital spherocytic anemia not in crisis, 2 patients with severe Mediterranean anemia, and 2 with sickle cell anemia failed to show antibody. In the crisis of 1 case of congenital spherocytic anemia an autohemolysin was temporarily found with the use of bovine albumin. Other cases of acquired hemolytic anemia (chemical, symptomatic, hypersplenic) failed to show antibody. Detection of antibody was of distinct value in the diagnosis of acquired hemolytic anemia of the "idiopathic" type. Its continued presence in such a case after splenectomy rendered the prognosis doubtful.

3. In the further differentiation of various types of familial and acquired cases, use was made of the red cell survival time. Parallel determinations of the survival time and of iso-antibodies showed a distinct correlation. Use of the anti-human globulin rabbit serum test to detect iso-antibody adsorbed to the red cell is cited as of further diagnostic aid.

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# CASE REPORT

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## HEMATOLOGIC OBSERVATIONS IN A CASE OF KALA-AZAR

By M. RACHMILEWITZ, M.D., K. BRAUN, M.D., AND A. DE VRIES, M.D.

THE hematologic changes generally seen in kala-azar are characterized by a striking reduction of red cells, white cells, and platelets. The mechanism of these profound changes might be diminished production in the bone marrow of all these elements, either due to metaplasia by proliferating reticulo-endothelial cells or due to some toxic inhibition by the infective agent, or an inhibitory effect upon the bone marrow of an enlarged spleen. Another explanation might be sought in an increased destruction of the blood elements by the hyperplastic organs of the reticulo-endothelial system, mainly the spleen. In this case the cell production in the bone marrow might proceed at a normal or even at an increased rate. The methods available for the elucidation of this problem are mainly limited to the red cell series. The type of the anemia, the number of reticulocytes, the composition of the bone marrow, and the quantitative determination of the bile pigments in blood and excreta might furnish valuable data for the understanding of the mechanism of the anemia. The variations of these data under the influence of specific therapy might be of particular importance. With these considerations in mind the studies on the following case were carried out.

### CASE REPORT

A young man, 18 years old, was admitted to our department on June 12, 1946. He was born in Palestine and had never left the country. Until one year ago he lived in an agricultural settlement, where he slept in a tent. During the last year he had lived in a town (Haifa). His past history was essentially negative. Four months previous to admission he began to complain of weariness and loss of appetite. One month later slight fever developed. At the same time he noticed that the whites of his eyes became yellowish. He was then examined for the first time and it was discovered that the spleen and liver were moderately enlarged. On April 9 he was admitted to the Municipal Hospital, Tel Aviv. During his two month's stay there he ran a subfebrile temperature and his spleen gradually increased in size to such a degree that it occupied the left half of the abdomen and caused abdominal distress. The liver also increased in size, but not considerably. The blood examination showed: hemoglobin 45% (Sahli), red cells 2.78 M./mm<sup>3</sup>, leukocytes 2800/mm<sup>3</sup>. The sedimentation rate was 20 minutes (Linzenmeier's method). The Takata-Ara, cephalin flocculation test and formol-gel reaction were strongly positive; the Weltmann test was IX. Repeated examination of the blood for malaria gave negative results. A careful search of the bone marrow punctate for *Leishmania donovani* bodies (including culture) also gave negative results. In spite of repeated blood transfusions and iron medication the patient's condition grew worse and he lost considerable weight.

On admission to our department, the patient was found to be in a poor nutritional state. His temperature was 38.2° C. He was very pale, and the sclerae were yellowish. There was no edema and there were no hemorrhages on the skin or mucous membranes. The pulse was 100, regular; blood pressure 100/70.

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A systolic murmur was heard at the base of the heart. Outstanding physical findings were confined to the abdomen, where a huge spleen occupied the entire left half of the abdomen, extending to the right of the umbilicus into the pelvis. It was of hard consistency and not tender; its surface was smooth. The liver was enlarged, its lower border being three fingerbreadths below the costal margin. It was also fairly hard, smooth, and not painful. Ascites was not detected.

**Laboratory Findings:** Urine: Albumin slightly positive, urobilinogen strongly positive, but no bilirubin, sediment negative.

Blood: Hemoglobin 8 gram %; red cells: 2.3 M./mm<sup>3</sup>; hematocrit 23 %; color index: 1.16; mean corpuscular volume: 100 cu. micra; mean corpuscular hemoglobin 34; mean corpuscular hemoglobin concentration 34%. Fragility test (NaCl solutions) 0.42-0.30%. Leukocytes: 1200/mm<sup>3</sup>. Differential count: neutrophils 28%, eosinophiles 2%, lymphocytes 54%, monocytes 16%. Reticulocytes: 1.5-5%. Thrombocytes: 110,000/mm<sup>3</sup>. Coagulation time: 7 minutes. Bleeding time: 3 minutes. Sedimentation rate: 7 minutes (Linzenmeier's method).

Blood chemistry: Urea 17 mg.%, glucose 82 mg.%. Total protein 9.40 g.%. Albumin 5.03 g.%, Globulin 4.35 g.%. Euglobulin strongly increased. Total fibrinogen 0.20 g.%. Formol-gel reaction positive within 4 minutes. Icteric index (Meulengracht) 25 units. Van den Bergh reaction: direct neg.

TABLE 1.—*Differential Bone Marrow Counts Before, During, and After Treatment*

	6/12/46	8/13/46	9/19/46
Myeloblasts. ....	4%	1%	1%
Promyelocytes.....	10%	2%	
Myeloc. Neut.....	23%	16%	17%
Myeloc. Eos.....	1%		
Metamyeloc. Neut.....	7%	14%	10%
Stab. Neut.....		20%	28%
Segment. Neut.....		9%	10%
Lymphocytes.....		2%	
Proerythroblasts.....	6%		
Erythroblasts.....	30%	8%	3%
Normoblasts.....	15%	16%	30%
Reticulum & Plasma cells.....	4%	2%	1%

ative, indirect positive. Takata-Ara 4 plus positive, cephalin flocculation test strongly positive, thymol test 25. Total cholesterol 84 mg.%, free cholesterol 56 mg.%, cholesterol ester 28 mg.%. Calcium 12.9 mg.%.

The examination of the bone marrow obtained by sternal puncture showed hyperplasia of the granulocytic and the erythroblastic tissues. The differential count showed myeloblasts 4%, promyelocytes 10%, neutrophile myelocytes 23%, eosinophile myelocytes 1%, neutrophile metamyelocytes 7%, proerythroblasts 6%, erythroblasts 30%, normoblasts 15%, plasma cells and reticulum cells 4%. Occasional erythroblasts in mitotic division were seen. Malaria parasites and *Leishmania* bodies were not found. On July 1 the bone marrow puncture was repeated and a culture for *Leishmania* was taken.

On the basis of the clinical picture characterized by hepatosplenomegaly, fever, leukopenia, and anemia, and in view of the negative results of examinations for malaria and *Leishmania*, the possibility of Hodgkin's disease was considered, and it was decided to irradiate the spleen with x-rays. During this treatment no improvement was noted and the spleen did not decrease in size. The number of red and white cells in the blood decreased. On July 17, i.e., on the seventeenth day after the culture of the bone marrow was taken, growth of *Leishmania* parasites was obtained. Thus the diagnosis of kala-azar, which was already suspected during his stay in the first hospital, was definitely proved.\* Therapy with stilbamidine

\* It is instructive to note that in some cases of Mediterranean kala-azar the number of parasites in bone marrow is too small to be detected in smears. But the diagnosis can almost always be established by culture. It is, therefore, important in all suspected cases not to rely on smears alone but to make cultures.

(4,4'-diamidino stilbene) intravenously was started on July 18. At first 0.5 mg. per kilo body weight was given daily; this dose was gradually increased to 1.5 mg./kg. All together 50 injections were administered. The response to the treatment was striking; after the eighth injection the temperature became normal and remained so. At the same time there was a rapid improvement in the patient's general condition. The spleen gradually decreased in size and at the end of the treatment its lower border was felt two fingerbreadths below the costal margin. The subicterus soon disappeared and the liver also diminished in size.

There was a gradual rise in the red and white blood cells which reached normal values at the end of the treatment. The percentage of the reticulocytes also gradually diminished. Subsequent bone marrow examinations revealed diminution of the immature white and red cells, the differential count becoming normal toward the end of the treatment (table 1). Bone marrow culture for Leishmania became negative.

The quantitative examinations of fecal and urine urobilinogen before and during the treatment with stilbamidine gave the following results. The daily excretion of urobilinogen in the feces amounted to 441, 539, and 569 mg./24h, on three examinations. These values decreased slowly during the treatment, and essentially normal amounts (22.6 mg.) were found at the end of treatment. The daily excretion of urobilinogen in the urine was markedly increased before treatment (52.5 and 55 mg./24h) and diminished gradually to almost normal values at the end of the treatment (5.9 mg.).

The only persistent pathologic findings were the changes in the serum proteins, the globulin fraction even slightly increasing after completion of the treatment. The formol-gel test was also still positive at this time.

#### COMMENT

The analysis of the hematologic data obtained before treatment strongly suggests that we were dealing with a hemolytic type of anemia, which was macrocytic and hyperchromic. The increased rate of red cell production was manifested by reticulocytosis ranging from 1.5 per cent to 5 per cent and by the hyperplastic bone marrow with a predominance of immature cells. Chatterie<sup>1</sup> reported the presence of a red bone marrow with a hyperplasia of the erythropoietic system and an increased number of "megaloblasts" in kala-azar. Also in experimental inoculation of monkeys with Leishmania a generalized hyperplasia of the bone marrow has been described (Shortt,<sup>2</sup> Meleney<sup>3</sup>). These findings are similar to ours and may be considered as a reaction to increased destruction of the blood cells. The increase of the bilirubin of the indirect type in the blood was another indication of augmented red cell destruction. More conclusive evidence was furnished by the quantitative determination of urobilinogen excretion in feces and urine, which was considerably increased before treatment and varied from 493 mg./24h to 594 mg./24h. These values are particularly high when the low red cell count is considered (Watson,<sup>4</sup> Miller, Singer, and Dameshek<sup>5</sup>). It is highly probable that the huge spleen was the most important site of red cell destruction. The elimination of the hyperactivity of the spleen by a chemotherapeutic agent affecting the organism responsible for the splenic enlargement, proceeded gradually and steadily in our patient. It was impressive to observe the parallelism between the reduction of the size of the spleen and the subsidence of the signs of its hyperactivity. The similar behavior of the red and white cells in regard to their reduced number in the peripheral blood before treatment, the hyperplasia of both elements in the bone marrow, and the effect of treatment causing a simultaneous rise of both, support the contention that the leukopenia was also due to increased destruction exceeding production. In case of the red cells their increased destruction could be objectively

proved by the determination of the urobilinogen excretion. No such objective evidence could be produced for a possibly increased destruction of the white cells. The estimation of uric acid excretion in the urine, the patient being on a special diet, did not reveal increased values. Although the uric acid content of the blood was within normal limits, this did not exclude the possibility of increased

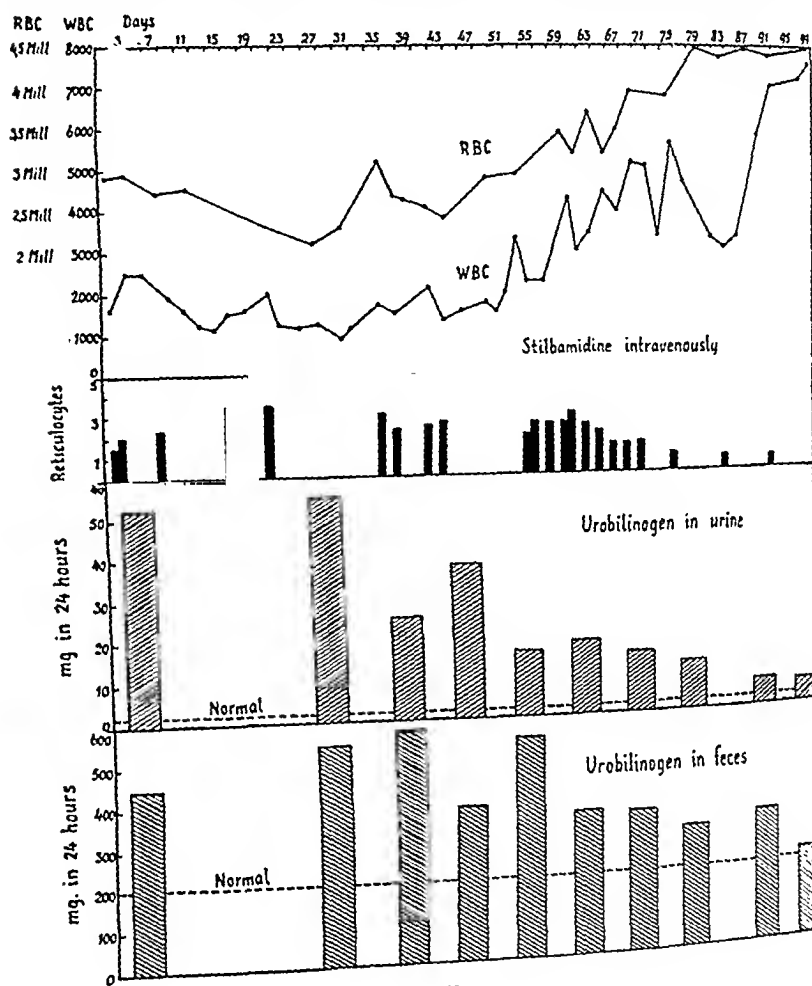


FIG. 1

leukocytolysis. Since the number of phagocytic cells in the spleen and other organs is known to be increased in kala-azar, it is reasonable to assume that phagocytosis was responsible for the increased destruction of all the blood elements. Adler<sup>6</sup> demonstrated indiscriminate phagocytosis of blood cells of all varieties by reticuloendothelial cells in kala-azar. According to Napier<sup>7</sup> the anemia in kala-azar may be due partly to excessive phagocytosis by the numerous macrophages. The conception of increased destruction of blood cells by a hyperactive phagocytosis in the spleen conforms with the observations of Doan et al.<sup>8</sup> and Muether et al.<sup>9</sup> in splen-

neutropenia. Using the supravital staining technic on fresh splenic parenchyma, Doan actually observed increased granulocyte inclusions along with red cells in the numerous reticulo-endothelial phagocytes. In these cases splenectomy resulted in normalization of the blood. The effects of the enlarged spleen on hemopoiesis may also be explained by a splenic hormone regulating bone marrow activity. The liberation of excessive amounts of this hormone by the enlarged hyperactive spleen may thus lead to interference with the normal production and delivery of blood cells. This explanation, rather than that of increased phagocytosis, is advanced by Dameshek<sup>10</sup> to account for the extreme neutropenia, thrombocytopenia and anemia of many cases with splenomegaly due to various causes, including chronic infection. Dameshek points to the lack of any clear-cut evidence indicating phagocytosis as the cause of the neutropenia and thrombocytopenia. It should also be stated that little direct evidence for the existence of hormonal activity has been produced thus far.

The analysis of the data obtained by the estimation of the urobilinogen excretion shows that the quantity of urobilinogen excreted in the urine was excessively high (52 mg. instead of 2 mg.). This fact points to a marked disturbance in the function of the liver in metabolizing the increased amounts of urobilinogen offered by the portal circulation. The liver in our case was definitely enlarged, and its disturbed function was also manifested by the positive cephalin flocculation test and the decrease of the cholesterol ester in the blood.

#### SUMMARY

The hematologic findings in a case of kala-azar under the influence of specific treatment are described. The type of the anemia, the hyperplastic bone marrow, the increased urobilinogen output before treatment, and the subsequent changes following treatment strongly suggest increased red cell destruction (most probably by phagocytosis) as the cause of the anemia.

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## EDITORIAL

### IMMUNOHEMATOLOGY

CHAUFFARD,<sup>1</sup> the great French clinician and clinical investigator, made suggestion in 1908 that a new specialty, "immunohematology," might be considered. Chauffard had discovered an hemolysin in the blood of a case of acute hemolytic anemia which he described under the designation of "acute hemolytic anemia." Shortly thereafter his Parisian colleagues, Widal, Abrami, and Brulé,<sup>2</sup> described autoagglutinins in cases of acquired hemolytic anemia and stated that this type of antibody was characteristic of the acquired cases as opposed to those of the congenital type.

The full merit of Chauffard's suggestion has only recently come to the fore notably through the great advances in the field of the Rh factors. Our own findings of iso-antibodies in the blood of cases of acquired hemolytic anemia revived Chauffard's original suggestion and led to the production of experimental hemolytic anemia with spherocytosis by the use of immune antibody.<sup>3</sup> Further studies demonstrated various types of hemolysins and agglutinins, the latter causing injury to the envelope of the red cell, which was then hemolyzed by such physical factors as mechanical trauma within the circulation.<sup>4</sup> Levine's concept<sup>5</sup> of iso-immunization with the Rh factor resulting in the development of anti-Rh agglutinin and subsequent iso-agglutination of the fetus' red cells appeared highly reasonable in the light of these considerations.

The intensive work with the Rh antibodies in hemolytic disease of the newborn has led to the uncovering of many new facts relating to iso-antibodies and thus indirectly to advances in the broader field of hemolytic anemia in general. Many of these advances were the subject of discussion at the recent Dallas-Mexico City meetings.<sup>6</sup> Demonstration of the so-called anti-Rh "blocking" antibody by the use of plasma, serum, or bovine albumin has led to the finding that similar antibodies are present in acquired hemolytic anemia in general.<sup>7</sup> In our own experience sera completely negative for iso-antibodies, using salt solution as a diluent, may show a distinct concentration of antibody with the use of bovine albumin.

What is more, the antiglobulin test by Coombs, Mourant, and Race<sup>8</sup> for demonstrating antibodies adsorbed to the red cell was found of distinct value by Hill and Haberman<sup>9</sup> in bringing out or "developing" the presence of an anti-Rh agglutinin in erythroblastosis foetalis. Boorman, Dodd, and Loutit<sup>10</sup> applied this test in cases of congenital and acquired hemolytic anemia and demonstrated a positive reaction in the acquired cases, the congenital cases giving a completely negative test. They concluded that in the acquired cases antibody was adsorbed to the red cell, thus confirming the hypothesis advanced originally by Dameshek and Schwartz.<sup>11</sup> Hill and Haberman<sup>12</sup> recently concluded that at least three orders of antibodies could be distinguished: (1) those readily demonstrable in salt solution, (2) those demonstrable in bovine albumin solution, plasma, or serum, but not in a salt solution medium, and (3) those demonstrable neither in salt solution nor albumin media but adsorbed to the red cell.

The Rh and Hr factors have proved to be of considerably greater complexity and interest than the first-described naturally occurring agglutinogens A and B. Hundreds of articles have appeared within the last few years dealing with their determination, their frequencies in various population groups, their hereditary and gene frequency relationships, their application to problems of disputed paternity, their occurrence in erythroblastosis foetalis and related conditions, their relationship to problems of blood transfusion, the various types of associated antibodies, etc.

Thus Chauffard's original idea of a special field of immunohematology has come to rapid fruition in the last few years. Undoubtedly still further advances will be made as the science of immunochemistry reaches its full development.

WILLIAM DAMESHEK, M.D.

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# ABSTRACTS

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## HEMATOPOIETIC TISSUES AND CYTOLOGY OF BLOOD CELLS

AN EXPERIMENTAL ANALYSIS OF HEMATOPOIESIS IN THE RAT YOLK SAC. *M. Block*. From the Department of Anatomy, University of Chicago, Chicago, Ill. *Anat. Rec.* 96: 289-332, 1946.

In the past many purely morphologic studies of the first circulating blood cells in mammalian yolk sacs have been undertaken to determine their nature. The present paper is unique in that it brings a new and interesting technic into this controversial field, the results of which now shed new light on the problem. Pieces of rat yolk sac from 11 to 17 day embryos were grafted into the anterior chamber of adult rat eyes. These were permitted to remain there for periods ranging from 1 to 30 days after transplantation. Grafts were vascularized by the third or fourth day and the debris incident to transplantation was cleared away. One of the first and most spectacular changes was the rapid and complete disappearance of primitive erythroblasts. The mechanism for this is not clearly understood. While this process was going on, large numbers of definitive erythroblasts, megakaryocytes, and myelocytes developed from mesenchymal cells. This finding is most striking since these cells normally form an inconsequential percentage of the yolk sac cells. From a theoretical point of view this is extremely important because it demonstrates that cells which develop primarily into primitive erythroblasts can unfold other potentialities by changing their environment. In addition to these experiments, Block also attempted unsuccessfully to influence embryonic erythropoiesis by administering various substances to the mother.

O.P.J.

OBSERVATIONS ON THE CHEMICAL CYTOLOGY OF NORMAL BLOOD AND HEMATOPOIETIC TISSUES. *G. B. White and E. W. Dempsey*. From the Department of Anatomy, Harvard Medical School, Boston, Mass. *Anat. Rec.* 96: 249-278, 1946.

For a number of years Wislocki and his associates have been investigating various tissues by means of physical and histochemical technics. The results of the present article should come as a breath of fresh air to some morphologic hematologists because Wislocki and Dempsey have investigated the lipids, glycogen, calcium, iron, mucoproteins, nucleoproteins, and various enzymes present in several hematopoietic tissues from 3 rhesus monkeys. Lipoid material was demonstrated in neutrophilic and eosinophilic granules. The latter, quite unlike those reported by Baillet and Kimbrough (*Anat. Rec.* [Suppl.]: 67, 1946), were described and pictured as solid spheres. Minute sudanophilic bodies were demonstrated only in neutrophilic leukocytes. From a histologic point of view, it is important to learn that nerve fibers actually accompany the arterial vessels in the marrow. While others have shown that the basophilia of lymphocytes is due to the presence of ribonucleoprotein, the present authors have demonstrated that this also obtains for erythroblasts, myeloblasts, and myelocytes. Tissue mast cells of the rat and human being were studied more extensively than other cells, and the results indicated that there is

species difference with respect to the presence of lipoidal material. The basophilic granules were not digested by either ribonuclease or hyaluronidase.

O. P. J.

HEMATOLOGY OF BLOOD SPOTS IN EGGS OF WHITE LEGHORN CHICKENS. *A. M. Lucas*. From the U. S. Regional Poultry Research Laboratory, Port Lansing, Mich. *Am. J. Anat.* 79: 431-472, 1946.

Blood spots in chicken eggs are of historical importance since they were first described by Aristotle. At present they should attract the attention of investigators working on leukemias since this may be the way in which avian leukosis is transmitted. Lucas studied blood spots in eggs present in the oviduct as well as those held for varying periods after laying. Because of Bloom's criticism of the dry smear method, Lucas relied chiefly on wet fixed smears, which in some instances were followed by the Feulgen reaction. Differential blood counts were made of the blood spots and blood of the hens. Lymphocytes and monocytes seemed more susceptible to damage than eosinophils and basophils. The latter were most resistant to degeneration. Macrophages in blood spots had their origin from fibroblasts of parent tissue rather than from vascular lymphocytes.

O. P. J.

SOME HISTOCHEMICAL ASPECTS OF THE MAST CELL WITH SPECIAL REFERENCE TO ALKALINE PHOSPHATASE AND CYTOCHROME OXIDASE. *C. R. Novack and W. Montagna*. From the Long Island College of Medicine, Brooklyn, N. Y. *Anat. Rec.* 96: 279-288, 1946.

Mast cells and their granules have been the subject of many morphologic and experimental studies. The present authors have attempted to determine their chemical nature and physiologic significance by using various histochemical techniques. The preputial gland and mesentery of the rat were the tissues selected for study because of the relatively high incidence of mast cells. Various technics were used to demonstrate the following: alkaline and acid phosphatase, cytochrome C, cytochrome oxidase, lipase, glycogen, peroxidase, free iron, lipoids, and aldehydes. The results showed that alkaline phosphatase was localized in the majority of mast cell granules. Acid phosphatase activity was present in only a few. The cytochrome C, cytochrome oxidase system was also present. Glycogen and lipid substances were absent.

O. P. J.

THE NATURE OF NEUTROPHILIC GRANULATION. *G. Discombe*. From the Pathological Department, St. Bartholomew's Hospital, London. *J. Path. & Bact.* 58: 572-573, 1946.

The present article confirms the observations made by Sheehan that leukocytic granules contain lipid (*J. Path. & Bact.* 49: 580, 1939). Discombe was unable to demonstrate by means of Baker's formol-calcium Sudan black technic a Golgi apparatus in the pale-staining clear area around which specific granules first appear. The difference in the appearance of neutrophilic granules stained with Leishman's stain and those colored with Sudan black appears to be due to the absorption of one of the azures on the surface of the lipid granule. The size, appearance, and distribution of these granules were indistinguishable from those seen in oxidase preparations.

O. P. J.

MITOTIC DIVISION AND DEGENERATION OF LYMPHOCYTES WITHIN CELLS OF INTESTINAL EPITHELIUM IN YOUNG AND IN ADULT WHITE MICE. *W. Andrew and J. M. Sosa*. From the Institute of Neurology and the Institute for Biological Sciences, University of Montevideo. *Anat. Rec.* 97: 63-98, 1947.

In previous articles Andrew and his associates have shown that lymphocytes migrate through epithelial cells of the duodenum in black mice of strain C 57. During this intracellular passage lymphocytes undergo certain changes. The present article reports the results of studying a different strain of mice in an attempt to confirm the previous findings and make additional cytologic observations. Twenty-three white mice ranging from 2 days of age to adults were studied. The youngest animals had fewer migrating lymphocytes in the villi than in the crypts of Lieberkühn. Activity in both of these locations increases with age. Some intracellular lymphocytes undergo mitosis, which may be aberrant. Lymphocytes on the apical side of epithelial nuclei are frequently twice the size of those on the basal side. The Golgi apparatus (Cajal technic) of lymphocytes in the apical region also becomes modified. At first it hypertrophies and later regresses by golgiorthexis and golgiolysis.

O. P. J.

LYMPHOCYTES IN THE INTESTINAL EPITHELIUM AND PEYER'S PATCHES OF NORMAL AND TUMOR-BEARING HAMSTERS. M. A. Kelsall. From the Biology Department, University of Colorado, Boulder, Colorado. *Anat. Rec.* 96: 391-410, 1946.

Kelsall has observed that small lymphocytes contain more desoxyribonucleoprotein (thymonucleic acid), as indicated by the Feulgen reaction, than the nuclei of several other somatic tissues. In view of the fact that Andrew and his associates have recently studied the occurrence of intracellular lymphocytes in intestinal epithelium, Kelsall considered it necessary to determine whether or not there were differences in the chemical composition of these lymphocytes and also those present in animals with a rapidly growing neoplasm. Tissues from 5 control hamsters and 5 with a subpannicular implanted mixed-cell sarcoma were studied. The Peyer's patch nearest the ileocecal junction was removed in each case with an adjacent piece of ileum. The vast majority (96 per cent) of the small lymphocytes within the columnar epithelial cells were located between the nucleus and the basement membrane. Neither the presence of a neoplasm nor the intracellular position of small lymphocytes altered the density of lymphocytic chromatin as compared with similar cells in Peyer's patches. Extranuclear particles of desoxyribonucleoprotein were scarce in the cytoplasm of epithelial cells. This has been interpreted to indicate that small lymphocytes in this location neither disintegrate nor act as trophocytes. Plasma cells were found in the stroma of villi; a few were also present in the basal cytoplasm of columnar epithelial cells. The author has suggested that perhaps small lymphocytes within the epithelial cells may synthesize basophilic cytoplasm and then migrate to the stroma of the villi. Many of the findings reported in this article are contrary to those of Andrew and Collings (*Anat. Rec.* 96: 445, 1946) and they may be explained on the basis of species difference as well as differences in the intestinal tract from which the material was obtained.

O. P. J.

LYMPHOCYTES WITHIN THE CELLS OF INTESTINAL EPITHELIUM IN MAN. W. Andrew and C. K. Collings. From the Departments of Anatomy, Southwestern Medical College and Baylor University College of Dentistry. *Anat. Rec.* 96: 445-458, 1946.

Duodena obtained from 10 autopsied human subjects were studied to determine whether or not lymphocytes entered epithelial cells and transformed there in a manner similar to that described previously in the mouse (*Anat. Rec.* 93: 251, 1945). The present authors reported that lymphocytes near the basal ends of epithelial cells are intercellular while those in the apical ends are intracellular. Lymphocytes in the latter position appeared to be undergoing degeneration changes as indicated by pyknosis and fragmentation. In spite of their intracellular position, lymphocytes may undergo mitosis. These findings, contrary to those made by Kelsall on the hamster, may be due to species differences.

O. P. J.

SIDEROCYTES IN MAMMALIAN BLOOD. R. A. M. Case. From the Department of Pathology, The Medical School, University of Birmingham, England, *Proc. Roy. Soc. London, Series B*, 133: 235-248, 1941.

Siderocytes, or red cells containing blue-green granules after staining with  $\alpha\alpha'$ -dipyridyl and potassium thiocyanate in dilute hydrochloric acid, were demonstrated in the blood of rat, dog, and mouse (and of other mammals by other observers). Siderocytes appeared most rapidly and in largest numbers in blood exposed to unfavorable conditions of storage and to chemical agents such as acetyl phenylhydrazine. Once the siderotic granules have been extruded, the erythrocyte appears to be normal morphologically but is probably more susceptible of phagocytosis. The siderocyte is probably the source of urinary siderotic granules described by Rous.

The author considers it likely that the siderotic change can take place only once in any given cell and that only a definite (and probably small) fraction of hemoglobin can be degraded by this mechanism. It is thought that the stainable, nonhematin iron of the siderotic granules is closely associated, if not identical, with the "easily split" blood iron described by other observers, and that this iron should be regarded as catabolic.

There is an obvious need for further studies on siderocytes, particularly in mammals with anemia associated with various forms of jaundice.

L. E. Y.

TISSUE METABOLISM STUDIES ON BONE MARROW. *C. O. Warren.* From the Cornell University Medical College, New York City. *Tr. N. Y. Acad. Sc. 8: 222-227, 1946.*

The in vitro culture of bone marrow cells is potentially a powerful tool for the study of the development of marrow cells, but to date technical difficulties have discouraged widespread use of this procedure. At best, such culture can be successfully maintained for only a matter of hours; during this time, however, studies of various types can be most instructive.

Warren employed the Warburg microrespirator to study the metabolic requirements of the cells of the bone marrow. His studies led to several conclusions:

1. Erythroid cells showed a predominance of respiratory (oxygen-utilizing) over glycolytic (lactic-acid-forming) activity. Myeloid cells, on the contrary, showed a predominantly glycolytic metabolism.
2. The normal metabolism of the myeloid cells was virtually identical with that of tumor cells.
3. Potassium arsenite was found to depress the (oxygen) respiration of normal and leukemic myeloid cells. Thio-uracil in high concentrations, but of the same magnitude as attained in the bone marrow of patients receiving the drug, also depressed myeloid respiration. It was postulated that this might be the mode of production of neutropenia by these drugs. It is of especial interest that pyridoxine was of no protective value in this regard in vitro.
4. When serum instead of saline was used as the substrate, both respiration and glycolysis were increased to twice their rates.

Details and further results with this and similar technics may be expected to provide fundamental explanations of certain problems of bone marrow cells, and of growth in general.

S. E.

## ANEMIA

THE ANEMIA OF THERMAL BURNS. *F. D. Moore, W. C. Peacock, E. Blakely, and O. Cope.* From the Department of Surgery of the Harvard Medical School, the Surgical Services at the Massachusetts General Hospital, and the Radioactivity Center of the Massachusetts Institute of Technology, Boston, Mass. *Ann. Surg. 124: 811-839, 1946.*

The anemia which develops in most patients after full thickness burns involving 10 per cent or more of the body surface has been intensively studied by the authors. Bone marrow activity was estimated by reticulocyte counts and by measuring the rate of utilization of radioiron. Transfusions of red cells containing radioiron were used in estimating total red cell mass of the burned patients. Following such transfusions the concentration of radioactivity in the peripheral blood remained constant under normal conditions, decreased if the donated cells were rapidly destroyed or if the patient's marrow became active, and increased if the patient's own cells were preferentially destroyed. Operative blood losses were computed from the concentration of acid hematin or of the radioactivity in the washings of sponges and drapes.

Three stages of the anemia of burns are described and it is explained that only one or two of the stages may be encountered in any one case. The first stage, lasting only a few days, is due to destruction of cells and is associated with a transient increase in erythrocyte fragility, the exact cause of which was not investigated by the authors. Depression of erythropoiesis is considered a likely cause of the second stage of anemia, observed during the first week or ten days following severe burns. The third stage, which may be separated from the second by a brief period of positive red cell balance, appears in the third or fourth week and is attributed to multiple causes including hemorrhage from the wound. The amounts of blood lost during excision of wounds and in changing dressings and the number of transfusions required to maintain a normal total red cell mass were surprisingly large. The roles of infection, iron deviation, and the "alarm reaction" are discussed, but no mention is made of the part that may be played by intravascular sludge formation. The need for early replacement of red cells is stressed and it is concluded that in controlling therapy there is no adequate substitute for serial measurements of total red cell volume.

All physicians who may be charged with the responsibility of treating severely burned patients would do well to read this impressive paper.

L. E. Y.

THE IMPORTANCE OF WHOLE BLOOD TRANSFUSIONS IN THE MANAGEMENT OF SEVERE BURNS. J. J. McDermid, E. F. Cadman, and J. Studder. From the Department of Surgery and the Division of Plastic Surgery, Columbia—Presbyterian Medical Center, New York, N. Y. *Ann. Surg.* 124: 332-353, 1946.

The authors analyze 10 cases having flame burns of from 30 to 80 per cent of their body surfaces. None of the 7 patients who survived the initial period of shock received adequate amounts of whole blood during the first week of illness to prevent the development of anemia. The data presented, however, serve to emphasize the urgent need for intensive, controlled whole blood transfusion therapy during the shock period. Whole blood is advocated in preference to blood substitutes because (1) it restores viscosity if plasma proteins have been depleted, (2) hemoglobin is a good source of protein, (3) the transfused red cell mass has a sparing action on body proteins by displacing plasma volume, (4) assimilation of protein given by mouth is improved after correction of anemia, and (5) because there may be a physiologic increase in blood volume when a large granulating surface is forming. It is admitted that the last-named consideration requires further study.

In addition to transfusions of whole blood, the authors recommend oral administration of sodium chloride solution in combination with lactate or bicarbonate in preference to sodium lactate solution alone.

L. E. Y.

FATAL APLASTIC ANEMIA FOLLOWING USE OF TRIDIONE AND A HYDANTOIN. F. F. Harrison, R. D. Johnson, and D. Ayer. From the Mary Imogene Bassett Hospital, Cooperstown, N. Y. *J. A. M. A.* 132: 11-13, 1946.

APLASTIC ANEMIA AND AGRANULOCYTOSIS FOLLOWING TRIDIONE. A FATAL CASE. R. P. Mackay and W. K. Gottstein. From the University of Illinois College of Medicine and St. Luke's Hospital, Chicago. *J. A. M. A.* 132: 13-16, 1946.

These two reports describe, for the first time, the occurrence of aplasia of the marrow following the use of tridione for convulsive disease. In the first case a 16 year old girl with grand mal was maintained on tridione and methyl-phenyl-ethyl hydantoin (a compound related to dilantin) for six months, when she rapidly developed aplastic anemia and died despite therapy. The second case was that of a 24 year old woman who had had petit mal since the age of 5. After receiving tridione and phenobarbital for a period of ten months, with good symptomatic response and no untoward effects, she suddenly developed marrow aplasia and died seventeen days later despite various attempts at therapy.

It is a fair assumption that in both instances the offending chemical was tridione (3,5,5-trimethyl-oxazolidine-2,4-dione). The cautious use of this material, as recommended by the authors, is certainly indicated.

S. E.

STUDIES ON MALARIAL PARASITES. IV. SOME OBSERVATIONS REGARDING THE AGE OF THE ERYTHROCYTE INVADDED BY *Plasmodium Vivax*. J. W. Ferrelle, J. G. Gibson, and W. C. Peacock. From the Harvard Medical School, Boston, Mass., and the Massachusetts Institute of Technology, Cambridge, Mass. *J. Inf. Dis.* 78: 180-182, 1946.

The advent of radioactive iron promises to provide a powerful tool for hematologic investigations. The present report demonstrates an ingenious mode of its application to the labeling of red cells.

It is known that radioactive iron given intravenously is incorporated into hemoglobin and begins to appear in the blood stream within twenty-four hours. That is, radioactive iron is taken up by erythrocytes only during the period of their formation. Hence, the radioactivity of a blood sample after a given dose of radioactive iron is a measure of the rate of addition of new erythrocytes to the blood stream. A patient who was inoculated with *P. vivax* was given radioactive iron intravenously, and blood was subsequently taken for erythrocyte counts, parasite counts, and parasite concentration. It was found that the radioactivity of the concentrates greatly exceeded the radioactivity of whole blood. Since radioactivity is limited to red cells formed after the injection of the radioactive iron, the concentrates

therefore contained a large number of young cells. The method of concentration, however, was for the malarial parasite; i.e., the concentrate contained largely parasitized cells. Hence, it could be concluded that the parasite preferentially invades the young red cell.

Of even greater importance than this result is the ingenuity of the method, which serves to demonstrate the tremendous potentialities of the use of radioactivity in fundamental hematologic investigations.

S. E.

**CONGENITAL HEMOLYTIC ANEMIA—A CASE REQUIRING EARLY SPLENECTOMY.** *E. Conrad and R. E. Schmidt.*

From the Department of Pediatrics, Duke University School of Medicine and Duke Hospital, Durham, N. C. *Am. J. Dis. Child.* 72: 731-733, 1946.

A case is reported of a white boy who developed classical evidence of congenital hemolytic anemia at the age of 17 days. Pertinent findings were anemia, jaundice, splenomegaly, hepatomegaly, microspherocytosis, reticulocytosis, nucleated red cells in the peripheral blood, and increased fragility of erythrocytes in hypotonic saline. The mother's blood also showed spherocytosis and increased osmotic fragility. Although detailed studies on the survival of donated cells were not made it is stated that the child's hemoglobin fell rapidly following transfusion. Splenectomy was performed at the age of 10 weeks with good response. It is implied that this is the earliest age at which symptoms and signs of congenital hemolytic anemia have been reported. Sections of the spleen are described as showing an increase of iron pigment and fibrosis in the sinusoidal tissues. No mention is made of the degree to which the splenic pulp was filled with red cells.

L. E. Y.

**MORPHOLOGICAL CHANGES IN THE RED CELLS IN RELATION TO SEVERE BURNS.** *A. Brown.* From the Royal Infirmary, Glasgow. *J. Path. & Bact.* 58: 367-372, 1946.

It was reported previously that when burns involve more than 15 per cent of the body surface, they are frequently accompanied by a moderately severe anemia. The present article extends observations along this line to include variations in the mean corpuscular volume, mean corpuscular diameter, and mean corpuscular average thickness in relation to osmotic fragility. In cases with the less severe burns microspherocytosis was present in the stained films, while cases with the more severe burns showed fragmentation and degeneration of red cells within a few hours after burning. Brown concluded that these morphologic changes were due to the direct action of heat on the cells.

O. P. J.

## POLYCYTHEMIA AND THE LIFE SPAN OF THE ERYTHROCYTE

**THE INCREASE IN HYPOXIA TOLERANCE OF NORMAL MEN ACCOMPANYING THE POLYCYTHEMIA INDUCED BY TRANSFUSION OF ERYTHROCYTES.** *N. Pace, E. L. Lozner, W. V. Consolazio, G. C. Pitts, and L. J. Pecora.*

From the Naval Medical Research Institute, Bethesda, Maryland. *Am. J. Physiol.* 148: 152-163, 1946.

These studies were undertaken in an attempt to determine the effect of artificially produced polycythemia on tolerance to high altitudes. One thousand cc. of red blood cells were transfused into each of 5 normal subjects during a period of four days. The mean hematocrit was increased from 46.2 to 58.3 per cent, an increase greater than had been expected, and believed to have been accounted for by hemoconcentration. No adverse symptoms attributable to the polycythemia were encountered, and there was definite increase in the tolerance of the subjects to hypoxia, as judged by the pulse rate during exercise under conditions of lowered oxygen tension. The exercise pulse rate dropped sharply following the transfusion of red cells and gradually increased during the following fifty days as the hematocrit decreased toward normal. It was estimated that the transfused subjects at an altitude of 15,500 feet had the same tolerance to exercise that the untransfused controls had at 10,300 feet.

The artificially induced polycythemia persisted for approximately fifty days, about half the time estimated for the maximum life of transfused red blood cells. This would indicate that either there had

been an increased rate of erythrocyte destruction or a decreased rate of new erythrocyte formation. The latter seemed more probable, since there was no increase in urinary pigment excretion, but a definite decrease in reticulocytes followed the transfusions. This suggested that normal erythropoiesis was inhibited to some degree by the presence of the injected erythrocytes.

J. F. R.

THE LIFE SPAN OF THE HUMAN RED BLOOD CELL. *D. Shemin and D. Rittenberg*. From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, N. Y. *J. Biol. Chem.* 161: 627-636, 1946.

Glycine labeled with  $N^{15}$  was fed to a man, resulting in the formation within the erythrocyte of heme containing a comparatively high concentration of the isotope. The level of  $N^{15}$  was followed in the circulating blood. The value rose rapidly to a high level, remained constant for about three months, and then fell. From this the average erythrocyte life span was calculated to be 127 days.

It was apparent that the heme pigment was a fixed molecule, not involved in the dynamic metabolic exchange shown to exist in most tissues. Its final disappearance coincided with the destruction of the erythrocyte. The figure of red cell life span by this method corresponds well to measurements by other reliable techniques.

C. A. F.

## BLOOD GROUPING AND THE Rh FACTOR

THE EFFECT OF CEREBRO-SPINAL FLUID ON THE INTERACTION BETWEEN Rh AGGLUTININS AND AGGLUTINOGENS. *R. Jakobowicz and L. M. Bryce*. From the Red Cross Blood Transfusion Service (Victoria Division) and the Walter and Eliza Hall Institute of Research in Pathology and Medicine, Melbourne. *M. J. Australia* 2: 740-743, 1946.

Cerebral spinal fluid of normal chloride and protein content was found to have an inhibitory action on the anti-Rh<sub>0</sub> agglutinating antibody. Agglutination of Rh<sub>0</sub> cells was completely inhibited when cerebrospinal fluid was used in place of saline as the diluent for the sera and the suspension media of the cells in three out of six sera, and the inhibition was partial but incomplete with three sera. This inhibitory effect was partially counteracted by the addition of human serum and was completely overcome by the addition of albumin. Fluids containing the inorganic and some of the organic constituents of cerebrospinal fluid failed to produce inhibition. Five of the fifteen samples of cerebrospinal fluid used were Rh negative individuals, so the inhibition of agglutination was not due to the presence of the specific substance and was probably dependent on a variable factor in the serum. Cerebrospinal fluids from other species exerted an inhibitory effect, as did abnormal human fluids of increased protein content. Since the inhibitory effect of cerebrospinal fluid is overcome by agents known to aid agglutination of cells by the blocking antibody, it is suggested that inhibition may be related to the conversion of the agglutinating antibody to a blocking antibody. It is not clear whether the presence of cerebrospinal fluid prevents the sensitization of cells by the anti-Rh antibody or fails to provide the necessary media for agglutination. Observations designed to detect sensitization of cells exposed to the cerebrospinal fluid anti-Rh serum mixture should settle this point.

R. S. E.

## HEMOGLOBINEMIA AND BILIRUBINEMIA

A FATAL HEMOLYTIC REACTION FOLLOWING TRANSURETHRAL RESECTION OF THE PROSTATE GLAND. A DISCUSSION OF ITS PREVENTION AND TREATMENT. *C. D. Greer and E. A. Webb*. From the Urological Division of the Department of Surgery of the University of Minnesota, Minneapolis, Minn. *Surgery* 21: 56-66, 1947.

This is the first report calling attention to the hazard of intravascular hemolysis in transurethral

prostatectomy. The authors report a case of fatal anurea and postulate that the distilled water used as irrigating fluid during the operative procedure caused hemolysis by gaining access to the blood stream. Similar observations have been made at at least two other clinics and provide ample evidence of the potential hazard of this operation when distilled water is used.

C. A. F.

PHYSIOLOGIC ICTERUS OF THE NEWBORN. *L. W. Freeman, A. Loewy, and A. Johnson.* Fed. Proc. 5: 30, 1946.

By the fifth day of life, infants generally show an increase in the serum bilirubin. It has been shown that the level of free fatty acids and soaps in the serum of animals and human beings increases after the ingestion of fat; and, according to the authors, are enough to account for hemolysis sufficient to cause a significant bilirubinemia. Furthermore, the diet of the newborn infant is stated to have a higher fat content than that of the fetus.

On the basis of these postulates, Freeman and his co-workers obtained umbilical cord blood from 50 newborn children and then divided them into three groups according to the (controlled) fat contents of their respective formulae. Their results were as follows:

	Serum bilirubin at birth	Serum bilirubin on 5th day
Control group (3.6% fat).....	1.36 mg. %	5.41 mg. %
Group 1 (1.8% fat).....	1.36 mg. %	4.21 mg. %
Group 2 (0.03% fat).....	1.36 mg. %	3.09 mg. %

The conclusion drawn from these results is that the amount of fat ingested plays some role in the production of neonatal icterus, by affecting the amount of hemolysis. This conclusion seems justified, although it would be desirable to have erythrocyte levels to judge the relationship between the occurrence of bilirubinemia and destruction of red cells. There are various causes for jaundice in the newborn; and even so-called "physiologic" jaundice is probably a heterogeneous group, due perhaps in some cases to the mechanism here postulated.

S. E.

## AGRANULOCYTOSIS AND LEUKOPENIA

SPHINGOMYELINS: THEIR ACTION ON BLOOD CELLS, PARTICULARLY LYMPHOCYTES; THEIR SHARE IN THE NUCLEINATE-LIKE ACTION OF THE ETHER-INSOLUBLE FRACTION OF BRAIN LIPIDS. *E. H. Tompkins.*

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tenn., and the Laboratory of Applied Physiology, Yale University, New Haven, Conn. Bull. Johns Hopkins Hosp. 63: 57-77, 1946.

The author has presented evidence previously to show that ether insoluble fractions of lipoids ("protagon") produced a leukopenia followed by a lymphopenia and granulocytosis, then a lymphocytosis following injection into rabbits. Sphingomyelin obtained by further fractionation of the above lipoids produced a reaction characterized by a lymphopenia and a moderate granulocytosis. The circulating lymphocytes began to decrease in numbers shortly after injection and reached the lowest in 5 to 7 hours later when the number varied from 53 to 80 per cent of the control value. The granulocytes reached a peak of increase 3 to 12 hours after injection, showing a 25 to 108 per cent increase over the control values. The number of monocytes varied in relation to the lymphocytes and the numbers of all three elements were back to the control level 12 to 15 hours after injection. Control observations with 5 per cent glucose failed to show similar reactions although egg lecithin produced results somewhat similar but lesser in degree. The author calls attention to the differences and similarities in results obtained with sphingomyelin and the parent material "protagon" and suggests that sphingomyelin is responsible for the lymphopenia produced by "protagon." The similarity of the reaction to sphingomyelins to that following injections of pituitary adrenotropic and adrenal cortical hormone is pointed out and the theory of reciprocal relationship between the number of circulating lymphocytes and granulocytes is discussed.

Blood was obtained from the tail and studied at weekly intervals.

Six weeks after hypophysectomy the total erythrocyte count had decreased from 8.35 million cells per cu. mm. to between 5.0 and 6.0 million cells; the hemoglobin from 10.4 per cent to 70 per cent. The control operated rats showed no change from normal. The rats on the starvation diet showed an increase in the total erythrocyte count and the hemoglobin level.

Reticulocyte counts, though variable, showed a decrease following hypophysectomy but remained normal in the other two groups of animals.

There were no changes found in the total white cell count or in the differential white cell count.

The bone marrows after hypophysectomy were hypoplastic. No differential counts were made on the bone marrows.

Hypophysectomy in adult rats produced a decrease in total erythrocyte count, a decrease in hemoglobin, a decrease in reticulocyte percentage, and a hypoplasia of the bone marrow. These results were not due to the surgery or the decreased food intake. There were no changes in the white cell counts.

R. C. C.

THE HYPOPHYSIS AND HEMOPOIESIS. O. O. Meyer, E. W. Thewlis, and H. P. Rusch. From the Department of Medicine, University of Wisconsin Medical School, Madison, Wis. *Endocrinology* 27: 932, 1940.

This paper is involved with attempts to prevent the development of the anemia which is induced by hypophysectomy with injections of various hormones.

Growth hormone from the anterior lobe of the pituitary increased the number of reticulocytes, but this increase was not accompanied by an increase in the total erythrocyte count or the hemoglobin level.

Injections of 0.4 mg. of thyroxine every second day into hypophysectomized rats produced a reticulocytosis. The total erythrocyte count and the hemoglobin showed a gradual decline, but this anemia was not so severe as after hypophysectomy and no treatment. Thyrotropic hormone, from the anterior lobe of the pituitary, showed similar results.

Adrenotropic hormone also produced a reticulocytosis but the total erythrocyte count and the hemoglobin decreased just as if no treatment had been given.

Following thyrotropic hormone and thyroxine treatment, the anemia of hypophysectomized animals was not so severe. It would seem that the atrophy of the thyroid gland, known to occur following hypophysectomy, might be one factor in the cause of this anemia.

R. C. C.

THE EFFECTS OF ENDOCRINES ON THE FORMED ELEMENTS OF THE BLOOD. PART I: THE EFFECTS OF HYPOPHYSCTOMY, THYROIDECTOMY, AND ADRENALECTOMY ON THE BLOOD OF THE ADULT FEMALE RAT. R. Craft. From the Department of Anatomy, Boston University School of Medicine, Boston, Mass. *Endocrinology* 29: 596, 1941.

The object of this paper was to compare the effects of hypophysectomy, thyroidectomy, and adrenalectomy on hemopoiesis in the adult female rat in an attempt to determine whether the effects produced by hypophysectomy were primarily due to the removal of that gland or secondarily due to the decreased activity in the thyroid and adrenal cortex known to occur following hypophysectomy.

Hypophysectomy produced, in 40 days, a decrease in the total erythrocyte count from the normal level of 8.20 million cells per cu. mm. to between 5.0 and 6.0 million cells. The hemoglobin dropped from 100 per cent to 70 per cent. Reticulocyte percentage dropped from 1.7 per cent to below 1.0 per cent in all cases. The total white cell count, although quite variable, showed a significant rise from an average of 8.8 thousand cells per cu. mm. to 15.8 thousand cells. There was no change in the differential white cell count.

Thyroidectomy produced the following results. The erythrocyte count decreased to 6.47 million cells in 90 days. The anemia was much more gradual in its development than that following hypophysectomy. The hemoglobin decreased slightly. The total white cell count remained normal but the differential cell count showed a lymphocytopenia and a neutrophilia.

Adrenalectomy produced the following results. (All animals were maintained on 1 per cent NaCl.) The erythrocyte count and hemoglobin both showed temporary decreases but then returned to normal levels. All other features remained normal.

The bone marrows were not studied.

These data indicate that the thyroid may play a role in the anemia which is induced by hypophysectomy but that the adrenal cortex does not. The possible roles of water balance, inanition, and posterior lobe removal are discussed.

R. C. C.

EFFECT OF SEX AND GONADOTROPIC HORMONES UPON THE BLOOD PICTURE OF THE RAT. *E. P. Vollmer and A. S. Gordon*. From the Department of Biology, Washington Square College of Arts and Sciences, New York University, New York City. *Endocrinology* 29: 828, 1941.

Testosterone propionate injections raised the erythrocyte count of hypophysectomized rats from the typical anemic levels to normal. The hemoglobin did not return to normal levels but did increase. Pregnant mare serum injections, known to stimulate the release of androgens from the testes, had similar effects. In both groups of rats a reticulocytosis and a bone marrow hyperplasia occurred.

Estradiol benzoate, on the other hand, had opposite effects. The anemia of hypophysectomized animals was increased. Injections of pregnant mare serum in the hypophysectomized female rats had no effect. Reticulocyte counts were low and the bone marrow exhibited a hypoplasia.

Pregnancy urine extracts produced no changes and progesterone injections gave inconsistent results. These data indicate that androgens can be classed as erythropoietic agents while estrogens cannot.

R. C. C.

EFFECTS OF HORMONES ON ERYTHROPOIESIS IN THE HYPOPHYSECTOMIZED RAT. *E. P. Vollmer, A. S. Gordon, and H. A. Charipper*. From the Department of Biology, Washington Square College of Arts and Sciences, New York University, New York City. *Endocrinology* 31: 619, 1942.

Adult male rats were hypophysectomized and the subsequent anemia allowed to develop. Attempts were made to eliminate this anemia with injections of (1) adrenal cortical extract, (2) desoxycorticosterone acetate, (3) prolactin, (4) crystalline thyroxine, and (5) thyroid powder.

Adrenal cortical extract: 5 rats were given 1 to 2 cc. of this extract daily for 5 weeks. No beneficial effects were obtained.

Desoxycorticosterone acetate: 6 rats were given daily injections of 1.0 mg. of this material for 40 days. A slight increase in erythrocyte count occurred. There was no change in reticulocyte counts or hemoglobin levels.

Thyroid powder plus desoxycorticosterone acetate: 7 rats were given 1.0 mg. of DOCA plus a ration containing 8.3 mg. of thyroid powder per Gm. each day. The latter was given orally. The erythrocyte count returned to normal levels. The hemoglobin increased "approximately to normal." The reticulocytes increased initially and then decreased. The bone marrow showed considerable repair.

Crystalline thyroxine: 8 rats were injected with daily doses of 0.01 to 0.03 mg. of thyroxine for 5 weeks. The erythrocyte count increased from 6.40 million cells per cu. mm. to 8.2 million cells. The hemoglobin also increased but the percentage increase was only half as great as obtained in the erythrocyte count. The reticulocyte response varied from no response to a slight response. The bone marrow gave the appearance of complete repair.

Prolactin: 4 rats were given daily doses of 0.5 to 1.5 mg. of this material for 40 days. These injections produced moderate gains in erythrocyte and hemoglobin levels and a fair repair of the bone marrow.

Of these hormones, thyroxine seems to have produced the most beneficial effects.

R. C. C.

EFFECT OF ANDROGENS ON THE BLOOD COUNT OF MEN. *E. P. McCullagh and R. Jones*. From the Cleveland Clinic, Cleveland, Ohio. *J. Clin. Endocrinology* 2: 243, 1942.

Twelve men were treated with testosterone propionate, methyl testosterone, or both combined. Eight were eunuchoids, 2 had hypogonadism, and 2 were sexually mature men. Studies were made on erythrocytes, hemoglobin, hematocrits, and leukocytes over periods varying from 1 month to 8 years.

With therapy, 7 of the 8 eunuchoids exhibited a rise in hemoglobin, erythrocyte, and hematocrit levels. These were decreased upon withdrawal of therapy.

Those patients showing a rise in the blood count also showed a rise in the basal metabolic rate.

These data showed that there was an increase in the BMR when androgen injections were given

to the human beings, indicating that androgens possibly stimulate hemopoiesis through increasing the metabolic rate.

R. C. C.

**THE EFFECTS OF IRON, COPPER, AND THYROXINE ON THE ANEMIA INDUCED BY HYPOPHYSECTOMY IN THE ADULT FEMALE RAT.** R. C. Crafts. From the Department of Anatomy, Boston University School of Medicine, Boston, Mass. *Am. J. Anat.* 79: 267, 1946.

This report deals with findings obtained in an attempt to determine why a severe anemia occurs after hypophysectomy.

This work revealed that the anemia induced by hypophysectomy was of the microcytic hypochromic type, which was accompanied by a hypoplasia of the bone marrow. Hypophysectomized rats were, accordingly, treated with ferrous sulfate, ferrous sulfate plus cupric sulfate, thyroxine, and ferrous sulfate plus cupric sulfate plus thyroxine.

Daily injections of 0.01 mg. of thyroxine, 0.5 mg. of ferrous sulfate, and 0.025 mg. of cupric sulfate for 30 days, followed by daily injections of 0.01 mg. of thyroxine, 2.0 mg. of ferrous sulfate, and 0.1 mg. of cupric sulfate for 20 days produced the best results. The combination of these three materials, injected into hypophysectomized adult female rats, maintained a normal erythrocyte count, completely prevented the microcytosis, practically prevented the hypochromia, not only prevented the hypoplasia of the bone marrow but produced a hyperplasia, and increased the number of erythroid elements in the bone marrow from the mean normal control level of 50.2 per cent to 59.5 per cent of marrow cells. The mean percentage of erythroid elements in hypophysectomized rats was 40.8.

The author has summarized the literature and pointed out that the materials which have been most beneficial in the hypophysectomized rat have been hormones which would stimulate the basal metabolic rate, i.e., crude pituitary preparations, thyroxine, androgens, and iron copper thyroxine combinations. Differential counts were made on the bone marrows, and photomicrographs of erythrocytes and bone marrows are included.

R. C. C.

**EFFECTS OF HYPOPHYSECTOMY, CASTRATION, AND TESTOSTERONE PROPIONATE ON HEMOPOIESIS IN THE ADULT MALE RAT.** R. C. Crafts. From the Department of Anatomy, Boston University School of Medicine, Boston, Mass. *Endocrinology* 39: 401, 1946.

Adult male rats were hypophysectomized, hypophysectomized and treated with 2.0 mg. of testosterone propionate, castrated, and castrated and treated with 2.0 mg. of testosterone propionate. Treatment was started immediately after surgery. Injections were given daily.

Hypophysectomy caused a severe microcytic hypochromic anemia, marked decrease in hemoglobin, hypoplasia of the bone marrow, a decrease in percentage of erythroid elements in the bone marrow, and an increase in the leukocyte count.

Androgen therapy in hypophysectomized adult male rats prevented the decrease in erythrocyte count, increase in total white cell count, and hypoplasia of the bone marrow; and partially prevented the hypochromia, the microcytosis, and decrease in the total number of erythroid elements.

Castration produced a slight decrease in erythrocyte and hemoglobin levels. These changes did not compare in severity with those following hypophysectomy.

Androgen therapy in castrated animals produced a hyperplasia of the bone marrow and increased the number of erythroid elements. Other factors were not studied in this group.

These data indicate that androgens are erythropoietic agents but that the loss of androgen via hypophysectomy is not the cause of the anemia in hypophysectomized animals.

R. C. C.

**COMPARATIVE STUDIES ON THE EFFECTS OF ESTRADIOL AND STILBESTROL UPON THE BLOOD, LIVER, AND BONE MARROW.** D. Castrodale, O. Bierbaum, E. B. Helwig, and C. M. MacBryde. From the Department of Medicine, Washington University School of Medicine, St. Louis, Mo. *Endocrinology* 29: 363, 1944.

Adult male and female dogs were treated with doses of stilbestrol ranging from 1.0 mg. to 100 mg. daily or doses of estradiol benzoate ranging from 1.66 mg. to 3.32 mg. Studies were made on the blood, liver, and bone marrow. The results obtained were as follows:

Blood: A sharp increase in the total white cell count occurred which was followed by a marked leu-

kopenia. In one case the leukocyte count went up as high as 133,000 cells per cu. mm. Differential counts showed that the neutrophils were the cells responding. The other cells remained at normal levels. A gradual decrease in erythrocyte and hemoglobin levels accompanied this rise in total white count. The blood platelets decreased in number to such an extent that thrombocytopenia followed.

**Bone marrow:** The bone marrow became hyperplastic, the myeloid elements being responsible. This was followed by areas of hypoplasia. No differential counts were made on the bone marrow.

**Liver:** The results were inconstant. Four dogs showed some fatty degeneration, 1 moderate central necrosis, and 2 showed no changes.

Compared by estrogenic potency, estradiol produced more rapid and more profound changes in the bone marrow and the blood than did stilbestrol.

R. C. C.

**THE EFFECT OF ENDOCRINES ON THE FORMED ELEMENTS OF THE BLOOD. PART II: THE EFFECT OF ESTROGENS ON THE DOG AND MONKEY.** R. C. Crafts. From the Department of Anatomy, Boston University School of Medicine, Boston, Mass. *Endocrinology* 29: 606, 1941.

Adult female dogs were treated with daily injections of either estradiol benzoate or stilbestrol. The severity of the response varied from animal to animal. A typical response was as follows:

An adult female dog was given injections of 5 mg. of stilbestrol for 27 days followed by 10.0 mg. for 7 days. This resulted in a sharp rise in the total white cell count to a peak of 54.3 thousand cells per cu. mm. in 23 days. This was followed by a rapid drop to 200 cells per cu. mm. by the 34th day, the day of death. Differential counts showed the neutrophils to be responsible for this rise. The erythrocyte count gradually decreased from 6.4 million cells to 4.0 million. Thrombocytopenic hemorrhagic purpura started on day 25.

The other dogs reacted in a similar manner except that they withstood the injections for longer periods, one lasting 122 days before it died.

This same treatment, with 10.0 mg. of stilbestrol, was given to adult female rhesus monkeys. This daily dose of estrogen, a fourfold dose per body weight, had no effect upon hemopoiesis in the monkey. With partial liver damage, imposed by oral administration of  $\text{CCl}_4$ , estrogens would produce an anemia in the monkey. At no time, however, was there a leukocyte response such as observed in the dogs.

This paper goes into detail on the normal hematologic figures for the monkey, comparing results obtained with those reported in the literature. The first blood sample obtained from a monkey, being an intractable beast, was very misleading. This first count showed a high white cell count with lymphocytes predominating. After a few weeks of training, the figures for the monkey were observed to be similar to those for the human being.

These data indicate that any estrogen in large doses is toxic to the bone marrow in dogs. Bone marrow studies were not included in this report.

R. C. C.

**INFLUENCE OF HORMONES ON LYMPHOID TISSUE STRUCTURE AND FUNCTION. THE ROLE OF THE PITUITARY ADRENOTROPIC HORMONE IN THE REGULATION OF THE LYMPHOCYTES AND OTHER CELLULAR ELEMENTS OF THE BLOOD.** T. F. Dougherty and A. White. Department of Anatomy, Yale School of Medicine, New Haven, Conn. *Endocrinology* 35: 1, 1944.

The object of this paper was to present evidence that the pituitary adrenotropic hormone is the factor which regulates the number of blood lymphocytes.

Single injections of adrenotropic hormone in rats, rabbits, and mice produced an absolute lymphopenia. This reaction occurred within a few hours after the injection. This response could not be elicited in adrenalectomized animals or in intact animals treated with pure protein. The authors thus claim that the lymphocyte response is specific to the hormone injection.

The total erythrocyte count and the hemoglobin levels showed a slight decrease.

These same responses could be elicited with adrenal cortical extract, adrenal cortical steroids in oil, corticosterone, or compound F of Wintersteiner.

These same authors, in other publications, have found a correlation between this decrease in lymphocyte number and an increase in serum protein, indicating the release of the protein from the broken-down lymphocytes.

R. C. C.

## LEUKEMIA AND LYMPHOMA

**NITROGEN MUSTARDS IN THE TREATMENT OF NEOPLASTIC DISEASE.** C. P. Rhoads. From the Committee on Growth, National Research Council, Washington, D.C. J. A. M. A., 131: 656-658, 1946.

**NITROGEN MUSTARD THERAPY. USE OF METHYL-BIS (BETA-CHLOROETHYL) AMINE HYDROCHLORIDE AND TRIS (BETA-CHLOROETHYL) AMINE HYDROCHLORIDE FOR HODGKIN'S DISEASE, LYMPHOSARCOMA, LEUKEMIA, AND CERTAIN ALLIED AND MISCELLANEOUS DISORDERS.** L. S. Goodman, M. M. Wintrobe, W. Darshek, M. J. Goodman, A. Gilman, and M. T. McLennan. J. A. M. A., 132: 126-132, 1946.

**NITROGEN MUSTARD THERAPY. STUDIES ON THE EFFECT OF METHYL-BIS (BETA-CHLOROETHYL) AMINE HYDROCHLORIDE ON NEOPLASTIC DISEASES AND ALLIED DISORDERS OF THE HEMATOPOIETIC SYSTEM.** L. O. Jacobsen, C. L. Spurr, E. S. G. Barron, T. Smith, C. Lushbaugh, and G. F. Dick. From the Department of Medicine, University of Chicago, Chicago, Ill. J. A. M. A. 132: 263-271, 1946.

These three reports summarize extensive investigations of the effects of the nitrogen mustards on neoplastic diseases, especially disorders of the hematopoietic system. A total of some 160 patients were treated. The official statement of Rhoads summarizes the results in a series of general statements:

1. The toxic effects of the methyl-bis compound are (a) local inflammation, if the material escapes from the vein; (b) nausea, vomiting, weakness, anorexia, and headaches, in a matter of hours; and (c) lymphopenia, neutropenia, anemia, and (rarely) hemorrhagic tendencies, often with thrombocytopenia.

2. The limits of therapy are as follows: (a) the nitrogen mustards do not cure; (b) the tumor regressions are transient, rarely extending beyond several months; (c) the chief toxicologic effect is hematopoietic damage, which at times may exceed damage to the tumor treated.

3. The effects of therapy are as follows: (a) In Hodgkin's disease, there may be dramatic dissolution of lymph nodes, with marked systemic improvement. The effect lasts from two weeks to eight months; subsequent relapses respond less and less to repeated therapy. In some cases, nitrogen mustards seemed to make radioresistant tumor masses more radiosensitive. (b) In lymphosarcoma, giant follicle lymphoma, chronic lymphatic leukemia, and chronic myelogenous leukemia, the methyl-bis compound seems to be approximately as effective as x-ray therapy. Goodman et al. reported occasional good results in lymphosarcoma. (c) There was no effect in the acute leukemias. (d) Rhoads et al., found encouraging results in anaplastic carcinoma of the lung. (e) Jacobson et al. found good results in polycythemia vera.

S. E.

**MULTIPLE MYELOMA. A REVIEW OF EIGHTY-THREE PROVED CASES.** E. D. Bayrd and F. J. Heck. From the Division of Medicine, Mayo Clinic, Rochester, Minn. J. A. M. A. 133: 147-157, 1946.

The authors review the symptoms and findings in 83 patients proven by marrow biopsy and/or autopsy to have had multiple myeloma. Treatment was uniformly of no value. Death occurred in from 1 month to 84 months after the onset of the disease, with an average of some 19 months. Tabulation of the principal abnormalities was as follows:

Pain.....	86%
Multiple bone lesions.....	78%
Elevated serum protein.....	73%
(In all but three cases, the ratio of albumin to globulin was reversed.)	
Renal dysfunction.....	61%
Marked rouleau formation.....	59%
Anemia.....	53%
Bence-Jones proteinuria.....	55%
Negative x-rays.....	12%
Myeloma cells in peripheral blood.....	10%

There was no prognostic value to the presence or absence of Bence-Jones proteinuria, or to the level of the serum proteins. It is of interest that, during the course of this study, Bence-Jones protein was found in 4 patients who did not have myeloma (1 with chronic lymphatic leukemia; 1 with carcinoma of the stomach; 1 with carcinoma of the kidney; and 1 with pulmonary tuberculosis and prostatitis).

S. E.

**STILBAMIDINE AND PENTAMIDINE IN MULTIPLE MYELOMA.** *I. Snapper.* From the Mt. Sinai Hospital, New York City. *J. A. M. A.* 133: 157-161, 1946.

Multiple myeloma has, in common with the otherwise unrelated disease kala-azar, an almost constant rise in the level of serum globulin. Because stilbamidine (diamidino stilbene; there is no antimony in this compound) is curative in kala-azar, Snapper decided to test its effect in myeloma, with remarkable results.

The effect of stilbamidine on the myeloma cells was to produce large cytoplasmic granules, which Snapper believes to consist of ribose nucleic acid. No such granules appeared in the other cells of the bone marrow. The effect of stilbamidine on the patient was to cause a disappearance of bone pain. The author believes that the drug arrests the proliferation of the myeloma cells for a period of time, and thereby eliminates the mechanism of bone pain in the disorder. Objective x-ray bone changes, in the direction of healing, occurred in some patients. There was no effect on the level of the serum globulin or on the excretion of Bence-Jones protein in the urine. The drug was ineffective unless the patient received, at the same time, a diet poor in animal protein—presumably because stilbamidine and arginine, which resemble each other chemically, may compete for the same chemical group in the myeloma cell. Caution is recommended in patients with renal disease. In a few cases a related compound, pentamidine, was also found useful.

The value of this discovery cannot be overemphasized. It may allow insight into the nature of the myeloma cell, with which stilbamidine apparently combines specifically; and if indeed the myeloma cell is the source of the abnormal serum globulins and the Bence-Jones protein found in the disease, further study of these abnormalities may prove profitable. It might also be of value to test the effect of stilbamidine on bone pain due to other disorders (presumably such pains would not be affected) and the effect of arginine (animal protein) on myeloma, in support of the author's mechanism of the action of stilbamidine. To the patient, stilbamidine and pentamidine may be symptomatic boons.

S. E.

**HISTOPATHOLOGY OF MONOCYTIC LEUKEMIA.** *P. A. Herbut and F. R. Miller.* From the Department of Medicine, Jefferson Medical College and Hospital, Philadelphia. *Am. J. Path.* 23: 93-124, 1947.

Like so many other articles on the subject of monocytic leukemia, the present one commences by stating that the first case was reported by Reschad and Schilling-Torgau in 1913. Downey (*Handbook of Hematology*) has thoroughly reviewed this subject and has pointed out why the so-called first case should be excluded from this group. Downey has also shown that monocytes in the leukemias may arise from the reticulo-endothelial system or from the myeloblast. In the present article histopathologic changes in 8 cases of leukemia have been studied, reported, and illustrated. Although Herbut and Miller were unable to demonstrate in all cases a definite hyperplasia of the reticulo-endothelial system resulting in a transformation of these cells into monocytes, they believe the reticulum acts as the precursor of monocytes.

O. P. J.

**MONOCYTIC LEUKAEMOID REACTION ASSOCIATED WITH TUBERCULOSIS AND A MEDIASTINAL TERATOMA.**

*A. Gibson.* From the Central Pathological Laboratory of Sector 8, E. M. S., St. Thomas's Hospital, Godalming. *J. Path. & Bact.* 58: 469-476, 1946.

Although much has been published about the various monocytic leukemias, the origin of monocytes is still open for discussion and little has been published about monocytic leukemoid reaction. The present case report focuses attention on the latter. Autopsy revealed that the bone marrow and splenic sinuses were filled with monocytes, whereas the alveolar exudate, stroma of the teratoma, and many smaller blood vessels contained numerous monocytes. No real leukemic infiltrations were found in the lungs, liver, kidneys, or suprarenals. Monocytes were present in relation to tuberculosis foci in the lung and liver.

O. P. J.

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# NEWS AND VIEWS

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At an informal gathering in Atlantic City on May 5, 1947 in conjunction with the meeting of the Society of Clinical Investigation, about 30 physicians interested in the field of hematology discussed the pros and cons of international and national societies of hematology. It appeared to be the consensus of opinion that:

1. An international society of hematology, already in process of formation, was of great potential value in fostering relationships between hematologists in various parts of the world.

2. The formation of a national society of hematology was inadvisable at this time. Numerous local and state hematologic societies or clubs were already in existence; the international society would take care of more formal meetings. A formally organized national society might tend to foster the further break-up of internal medicine into numerous small, highly specialized compartments to the detriment of both internal medicine and hematology. It was to the advantage of the physician in hematology to remain in close contact with the broader fields of internal medicine and pathology.

3. The desire of individuals interested in the same general field to convene could best be carried out, at least for the present, by the formation of a highly informal "blood club" or "hematology club," to meet in conjunction with the annual Atlantic City meetings of the Society for Clinical Investigation and the Association of American Physicians. A program similar to that carried out by the Central Society for Clinical Research in Chicago could be provided. This consists of an annual dinner followed by a round-table discussion of a series of selected topics.

4. The first (tentatively) scheduled meeting would be held on Sunday evening, May 2, 1948, Atlantic City, invitations to be sent in due course to all those physicians who have shown an interest in the field of hematology.

Those present at the informal meeting above referred to were as follows:

Benjamin Alexander, Boston  
Howard Alt, Chicago  
Frank Bethell, Ann Arbor  
William Dameshek, Boston  
C. S. Davidson, Boston  
A. DeVries, Jerusalem  
Charles Doan, Columbus, Ohio  
G. Emerson, Boston  
Ray F. Farquharson, Toronto  
Claude Forkner, New York  
Paul Fouts, Indianapolis  
Willis Fowler, Iowa  
J. J. Groen, Amsterdam  
R. Heinle, Cleveland  
John Lawrence, Rochester

Stacy Mettier, San Francisco  
Leo Meyer, Brooklyn  
O. O. Meyer, Madison, Wis.  
F. R. Miller, Philadelphia  
Carl Moore, St. Louis  
Savas Nittis, New York  
E. E. Osgood, Portland, Oregon  
Joseph Ross, Boston  
Wayne Rundles, Durham, N. C.  
L. Tocantins, Cleveland  
Louis Wasserman, New York  
Charles H. Watkins, Rochester, Minn.  
Maxwell M. Wintrobe, Salt Lake City  
L. E. Young, Rochester, N. Y.

In addition<sup>1</sup> the following expressed regrets at being unable to attend the meeting but in most instances wished to be included in any future society developments:

Alan Berstein, Baltimore  
 Gurth Carpenter, Los Angeles  
 W. B. Castle, Boston  
 L. F. Craver, New York  
 Elmer DeGowin, Iowa  
 Louis K. Diamond, Boston  
 Clement Finch, Boston  
 Russell L. Haden, Cleveland  
 Hale Ham, Boston  
 J. M. Hill, Dallas  
 Raphael Isaacs, Chicago  
 O. P. Jones, Buffalo  
 R. R. Kracke, Birmingham

Philip Levine, Raritan, N. J.  
 Eugene Lozner, Boston  
 G. R. Minor, Boston  
 Fred Pohle, Madison  
 Paul Reznikoff, New York  
 N. Rosenthal, New York  
 S. O. Schwartz, Chicago  
 Tom Spies, Birmingham  
 Maurice Strauss, Boston  
 C. C. Sturgis, Ann Arbor  
 Laskey Taylor, Boston  
 C. J. Watson, Minneapolis  
 Bruce Wiseman, Columbus, Ohio  
 Ernest Witebsky, Buffalo

Because of the greatly increased interest in the blood groups and related matters, the addition to BLOOD of a new section dealing with problems in immunohematology and transfusions is contemplated; we would appreciate receiving the comments of interested readers regarding this proposed innovation.

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## BOOK REVIEW

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*Human Genetics.* By REGINALD R. GATES. The Macmillan Company, New York. 2 vols. Pp. 1518, 1946. \$15.00

This book in two volumes is a review and compilation of accumulated knowledge in the field of human genetics. As such it is valuable both for its presentation of known genetic traits and also as a convenient starting point for further research. The unusually extensive list of references and the detailed index make this book eminently usable. The material is presented from the broad view of the biologist and introduces corollary evidence from other fields. In general, attention has been focused on the transmission of markedly abnormal conditions. Gates points out that standard genetic symbols (chap. 1) and methods of presentation should more generally be adopted for human heredity studies.

Three chapters will be of special interest to hematologists. The chapter on "Hemophilia and Related Hereditary Conditions" necessitates a fundamental revision of current views, as it shows (1) that heterozygous females (not very infrequently) are partial bleeders; (2) that hemophilia, while generally sex-linked, is (like other abnormalities) dominant in occasional families and probably recessive in rare cases. Sex linkage should therefore not be regarded as an essential diagnostic feature of hemophilia, although this is its usual method of inheritance. Several other types of bleeders are now known, including a dominant sex-linked form in which females are severely affected, as well as dominant pedigrees of epistaxis (nosebleeds), telangiectasis, and capillary fragility.

A table is presented which classifies fifteen inherited hemorrhagic conditions. A discussion of these diseases follows, with citation of original papers.

The second chapter of special interest is entitled "Other Inherited Diseases and Abnormalities of the Blood System." With regard to elliptocytosis (ovalocytosis), it is pointed out that "elliptical red blood

cells in man may be regarded as a reversion or rather the persistence of an embryonic condition" due to a single gene. Sickle cell anemia, stated as fundamentally an abnormality of erythropoiesis, is inherited as a Mendelian dominant. "Thalassemia" is reported as caused by a gene which is a "partial dominant, sublethal in the homozygous (major) condition." The following conditions and their heredity are also discussed in this chapter: acholuric jaundice, eosinophilia, Pelger's nuclear anomaly of the leukocytes, pernicious anemia, hypochromic anemia, methemoglobinemia, Felty's syndrome, high blood pressure, arteriosclerosis, hypotension, Hodgkin's disease, myelogenous osteopathy, varicose veins, thromboangiitis obliterans.

The third chapter of special interest deals with the blood groups and presents the inheritance of the ABO blood types as well as their possible origin and distribution. The inheritance of the secretor factor, the MN factor and Rh factors, is given in considerable detail.

Conclusions reached by workers in human genetics present puzzling differences when compared with experience in other animal material and plants. For example, in human genetics the former sharply drawn distinction between dominant and recessive is said to break down completely, and a sliding scale from strict dominance to recessive characters showing incomplete penetrance (many normal overlaps) occurs. Another odd feature is "anticipation"; this refers to the progressively earlier appearance of an inherited condition in successive generations. Also, numerous cases are known in human genetics in which the same abnormality of character is inherited in two or three different ways in different pedigrees, dominant, recessive, and recessive sex-linked being the usual types. The reviewer believes that increasing knowledge will obviate what seem to be conflicts between human and other animal genetics.

The dissatisfactions engendered by this book can be ascribed not so much to the presentation as to our present state of knowledge.

The field of human genetics is now in what might be called the encyclopedic state (a period concerned largely with the recognition and cataloguing of inherited characters). Progress now depends on the recognition of heritable characters which can be easily classified, are simply inherited, and are widely dispersed through the general population. A larger series of such genes such as ABO blood types and PTC tasters which could be used as markers for more of the 24 pairs of human chromosomes would give human genetics a tool it sadly lacks. The doctor who neglects heredity loses much material which would enable him to interpret his patient's disease in relation to the family history. Increasing knowledge of human heredity foreshadows the time when part of every research group and hospital staff will be specially trained in this field.

# BLOOD

## *The Journal of Hematology*

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### THE ERYTHROPOIETIC ACTIVITY OF CHOLINE CHLORIDE IN MEGALOBLASTIC ANEMIAS

By L. J. DAVIS, M.D., AND ALEXANDER BROWN, M.D.

#### INTRODUCTION

MOOSNICK, Schleicher, and Peterson<sup>1</sup> reported in 1945 that the administration of choline chloride resulted in the hematological remission of a case of pernicious anemia refractory to parenteral liver therapy. This action, attributed to choline, appeared to merit further study since, if it were confirmed, it would be desirable not only to assess the value of choline in the therapy of anemia but also to explore its possibilities in the elucidation of the etiology of megaloblastic anemias, especially those refractory to parenteral liver therapy. The present investigation was therefore undertaken with the object of observing the erythropoietic activity of choline in various types of megaloblastic anemia.

Before describing our findings, it may not be out of place to recapitulate the observations of Moosnick et al.<sup>1</sup> Their patient, a man aged 61 years, had been successfully treated for several years with intramuscular injections of liver extract but eventually relapsed, despite high dosage of refined liver extracts, and developed sensitivity to liver extracts, both refined and crude. When admitted to the hospital he was suffering from severe jaundice. The red cell count was 3.87 M., the sternal marrow showed megaloblastic erythropoiesis but was hypoplastic with fatty metamorphosis. Liver biopsy showed acute hepatitis and moderately severe fatty changes.

Choline chloride was administered intravenously in a daily dose of 1 gram for 16 days and was followed by a reticulocyte response of 5.5 per cent on the third day and a progressive rise in the red cell count. At the end of the period of treatment the peripheral blood status was normal, the patient's general condition improved, and the sternal marrow picture restored "toward normality." The patient was subsequently treated with ventriculin but died of pneumonia some months later, at which time autopsy examination revealed that the liver was practically normal. The authors suggest that in their patient an adequate amount of hematopoietin was stored in the liver but not elaborated in sufficient amounts on account of the fatty state of this organ and that this dysfunction was corrected by the administration of choline. Although there was no direct evidence of choline deficiency, it was thought that this was present on account of the conspicuous fatty changes in the liver and sternal marrow.

Although the case recorded by Moosnick et al.<sup>1</sup> seems to be exceptional in that

From Glasgow Royal Infirmary, Glasgow, Scotland.

it was complicated by severe hepatic disease, it appeared to us that, if their hypothesis were correct, the refractoriness to parenteral liver therapy displayed by certain other types of megaloblastic anemia might also result from hepatic dysfunction amenable to choline therapy. It also seemed possible, in view of the effect of choline on intestinal absorption (Frazer<sup>2</sup>), that choline might exert a favorable influence on megaloblastic anemias associated with defective intestinal function.

### METHODS

The clinical material studied by us consisted of 10 cases of macrocytic anemia, in all of which erythropoiesis, as revealed by sternal puncture, was megaloblastic. The cases are considered to be representative of five types of megaloblastic anemia, namely: (1) Addisonian pernicious anemia, (2) megaloblastic anemia of pregnancy, (3) megaloblastic anemia of the sprue syndrome, (4) nutritional megaloblastic anemia, and (5) refractory megaloblastic anemia of uncertain etiology.

The choline chloride employed was a commercial preparation (British Drug Houses) and was administered either orally dissolved in water or intravenously dissolved without previous heating in sterile normal saline. The daily dosage varied from 3 to 10 grams when given orally and from 1 to 10 grams when given intravenously. No side effects accompanied the oral medication. Unpleasant reactions were, however, apt to result from the intravenous administration of choline. When the dose was limited to 1 gram given slowly in a 5 per cent solution, these reactions were minimal, but they constituted a major objection to the giving of large doses. Doses of 5 or 10 grams were given in a 1 per cent slow drip infusion over periods exceeding three hours but were usually accompanied by unpleasant manifestations such as tachycardia, nausea, vomiting, sweating, abdominal discomfort, and depression. No significant reduction in blood pressure was noted, however. On account of these effects it was not feasible to continue high dosage intravenous therapy for more than three or four days.

The liver extracts were from batches known to be potent in cases of Addisonian pernicious anemia. The proteolyzed liver was the commercial preparation, "hepamino," given by mouth in divided doses, totaling approximately 36 grams daily. The oral liver extract was Armour's or Evan's preparation given in doses of 45 or 60 cc. daily.

The food, during the periods of observation, consisted of ordinary hospital diets containing relatively small quantities of first-class protein and no vitamin supplements.

The hematological technic calls for no special description other than to remark that the hemoglobinometers were standardized so that 100 per cent was equivalent to 14.8 grams of hemoglobin per 100 cc. The sternal puncture procedure was as described by Davis, Davidson, and Innes.<sup>3</sup> The liver biopsies were performed by the needle method (Sherlock<sup>4</sup>).

### CASE HISTORIES

#### A. ADDISONIAN PERNICIOUS ANEMIA

*Case 1.* Mrs. C., aged 71, was admitted to the hospital on August 12, 1946, with a history of symptoms attributable to anemia which had become progressively disabling during the previous three months.

The earlier medical history is not significant. She was a well-nourished woman displaying marked pallor and a slight degree of jaundice. The tongue was pale and slightly atrophied. No enlargement of the liver, spleen or lymph glands was detected and no signs of neurological complications were evident.

Laboratory findings: R.B.C., 1.48 M.; hemoglobin, 40 per cent; color index, 1.3; M.C.V., 117 cu. microns; reticulocytes less than 1 per cent; W.B.C., 2,800 per cu. mm. Stained blood film: typical macrocytic picture with poikilocytosis and anisocytosis. Sternal marrow megaloblastic erythropoiesis, hypercellular with no excessive fat. Gastric analysis: histamine-fast achlorhydria. Liver biopsy: no obvious histological abnormality, glycogen content of cells was normal and there were no gross fatty changes. Urine: urobilinogen was present.

Treatment and progress: Choline chloride in daily doses of 10 grams was given intravenously for three days but had to be discontinued on account of severe side effects. During the following eight days choline

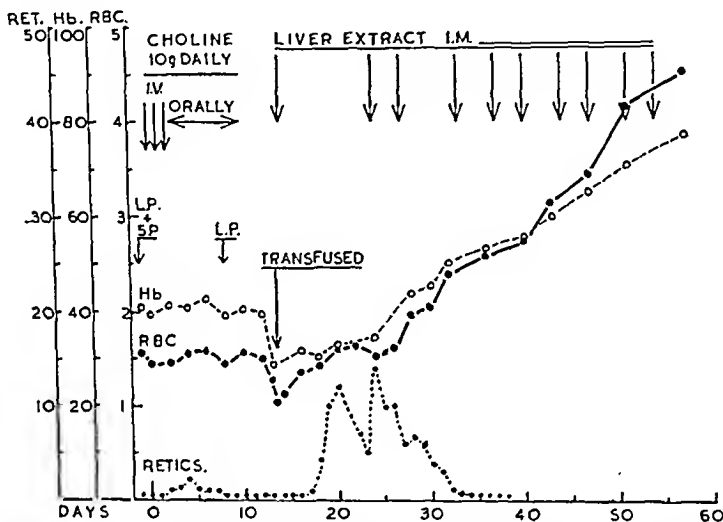


FIG. 1. A case of Addisonian pernicious anemia. Choline given by mouth and intravenously was without significant erythropoietic effect. The case responded satisfactorily to parenteral liver therapy. (In this and the succeeding figures, "S.P." denotes sternal puncture, and "L.P.," liver puncture.)

was given orally in divided doses aggregating 10 grams daily. On the fourth day after commencing choline therapy the reticulocyte count rose to a maximum of 2.5 per cent, but was not accompanied by any rise in the red cell count which, on the contrary, fell to under 1 million on the thirteenth day. This fall was accompanied by a deterioration in the patient's general condition requiring a blood transfusion.

The patient was subsequently given refined liver extract ("neo-hepatex") parenterally, to which she responded satisfactorily, as shown in figure 1, with subsequent maintenance of a normal blood picture.

**Case 2.** Miss D., aged 45, was diagnosed as a case of Addisonian pernicious anemia in 1944 and treated with parenteral liver therapy to which she responded satisfactorily. Later, however, she failed to continue treatment and was admitted to the hospital on February 19, 1946, in a severely anemic state. No noteworthy clinical findings were present other than those of severe anemia and a very slight degree of icterus. No enlargement of the liver, spleen, or lymph glands was detected and no signs of neurological involvement were present.

Laboratory findings: R.B.C., 0.7 M.; hemoglobin, 20 per cent; color index, 1.4; M.C.V., 143 cu. microns; reticulocytes, less than 1 per cent; W.B.C., 2,800 per cu. mm. Blood film: macrocytosis, poikilocytosis, and marked anisocytosis. Sternal marrow: megaloblastic, hypercellular, no excess of fat. Gastric analysis: histamine-fast achlorhydria. Urine: urobilinogen present. Liver biopsy was not performed.

Treatment and progress: Intramuscular injections of a refined liver extract ("anahaemin"), totaling 12 cc. in twelve days, resulted in a suboptimal reticulocyte response but in a sustained rise in the red cell count (fig. 2), until a level of 3,100,000 red cells was reached. Thereafter the red cells remained stationary for seven days and then fell to 2.7 M. Choline was then given intravenously in a daily dose of 1 gram for eleven days and was accompanied by a rise in the red cell count to 4.05 M. (fig. 2).

The patient has since been maintained satisfactorily with parenteral liver therapy.

Case 3. Mrs. M. S., aged 65, first developed anemia at the age of 59 and was given injections of liver extract with symptomatic improvement. Since this treatment was not maintained regularly, she was never completely free from symptoms and suffered constantly from a feeling of weakness and from undue dyspnea on effort. During the month prior to admission these symptoms became more severe.

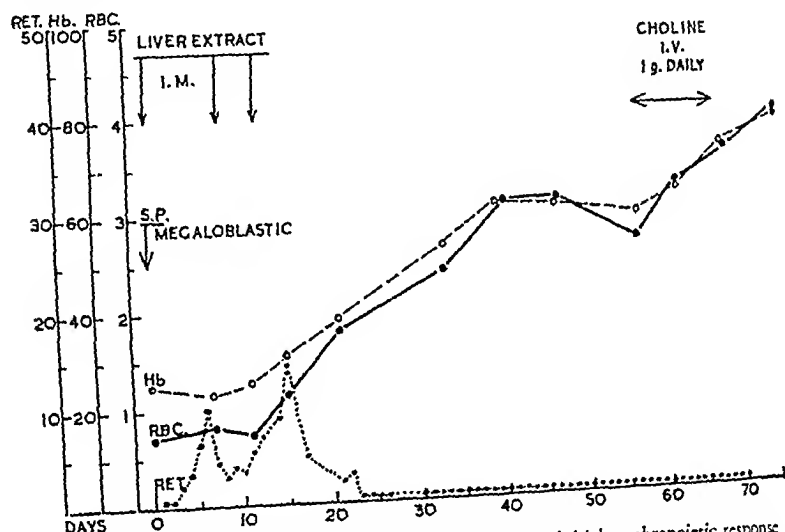


FIG. 2. A case of Addisonian pernicious anemia showing an initial erythropoietic response to injections of liver extract and a secondary rise in the red cells following the administration of intravenous choline.

On admission to hospital on November 8, 1946, she displayed the usual manifestations of severe anemia. Jaundice was absent, however, and no enlargement of liver, spleen, or lymph glands was detected, nor was there evidence of neurological disease.

Laboratory findings: R.B.C., 1.3; hemoglobin, 40 per cent; color index, 1.5; W.B.C., 4,600 per cu. mm.; reticulocytes, less than 1 per cent. Stained films showed marked macrocytosis, anisocytosis, and poikilocytosis. Sternal marrow: megaloblastic, hypercellular with no excess of fat. Gastric analysis histamine-fast achlorhydria. Liver histology: a biopsy performed twenty days after the first injection of liver extract and nine days before administration of choline showed no abnormalities other than a slight degree of patchy fatty degeneration. A second biopsy seventeen days after the institution of choline therapy showed no fatty change or other abnormality. The serum colloidal gold and the plasma alkaline phosphatase were within normal limits shortly after admission to hospital.

Treatment and progress: After an observation period of five days a single injection of liver extract ("hepastab" 4 cc.) was followed by a reticulocyte response of 18 per cent and a rise in the red cell count to 3.02 M on the twenty-fourth day. Thereafter the red cells fell to 2.75 M. on the thirtieth day. Further injections of liver extract were deliberately withheld in order to provide an opportunity of observing the effect of choline. Choline chloride was given intravenously in daily doses of 1 gram for twelve days and then by mouth in doses of 15 grams daily for a further period of fourteen days. Reference to figure 3 will show that the administration of choline was accompanied by a rise in the red cell count to 3.7 M.,

although no significant reticulocyte response was observed. The regeneration of red cells was not maintained, however, despite the continuation of oral choline therapy, and eventually, after withdrawal of choline, the red cells fell to 2.9 M. Parenteral liver therapy was then resumed with a consequent reticulocyte response of 7 per cent and a rise in the red cell count which eventually attained a normal level.

#### COMMENT ON CASES 1, 2, AND 3

In case 1, an example of untreated Addisonian pernicious anemia, choline was without significant effect. It will be noted that the substance was given in daily doses of 10 grams intravenously for three days and orally for seven more days. Subsequent parenteral liver therapy resulted in satisfactory red cell regeneration, although the reticulocyte response was suboptimal.

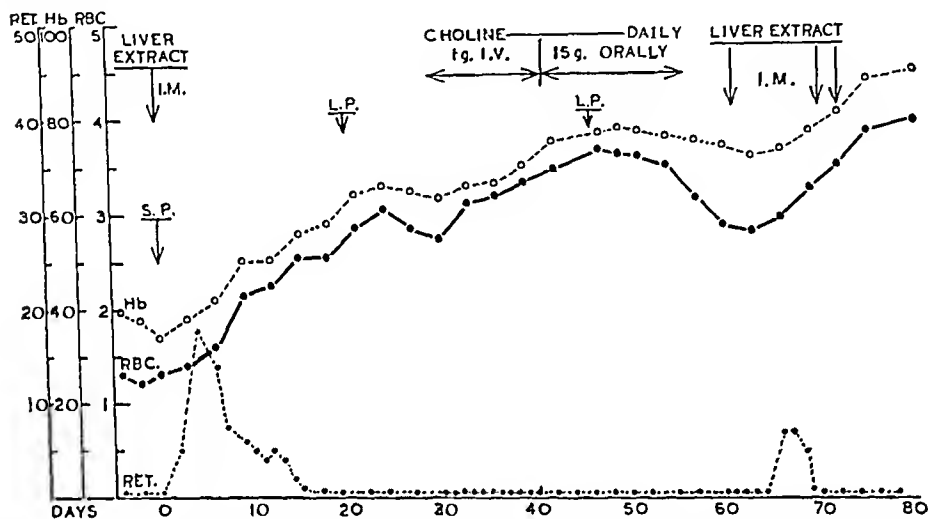


FIG. 3. A case of Addisonian pernicious anemia. An injection of liver extract resulted in an erythropoietic response. The subsequent administration of choline was followed by a secondary rise in the red cells which, however, was not maintained.

In cases 2 and 3, treatment was initiated by injections of liver extract which resulted in regeneration of red cells although the reticulocyte responses were suboptimal. When the hematopoietic effect of the liver extracts was apparently exhausted and the red cell count was falling, the administration of intravenous choline in both cases resulted in a further rise in the red cell count. Although no reticulocyte response to choline was observed in either case, it is reasonable to assume that the red cell response is attributable to the administration of choline.\*

\* Since submitting this paper for publication, we have observed the effect of choline chloride in 3 further cases of classical Addisonian pernicious anemia. In each case the choline was given subsequent to an initial hemopoietic response to a single injection of liver extract, after the red cell count had commenced to fall. The dosage was 1 gram daily for a period of five to seven days; the route was intravenous.

One case showed no significant response, but in the other 2 cases the administration of choline was followed by secondary but temporary rises in the red cell counts and hemoglobin levels similar to that described in cases 2 and 3 in the text. The magnitude of these secondary rises was from 2.6 M. to 2.95 M. in the one case and from 2.87 M. to 3.51 M. in the other.

It is noteworthy that in case 3 choline by mouth was not effective in maintaining a continued rise in the red cells.

#### B. MEGALOBLASTIC ANEMIA OF PREGNANCY

*Case 4.* Mrs. D. D., aged 36, developed a severe anemia during the later stages of pregnancy. She received injections of liver extract without effect and blood transfusions before and after a premature delivery. During the three months immediately following her delivery numerous injections of liver extracts (anahaemin) resulted in no improvement in her anemia. At this time, April 15, 1946, she came under our care. She was a pale, somewhat poorly developed woman but not ill nourished. Apart from manifestations of anemia, no noteworthy clinical findings were present. There was no evidence of jaundice.

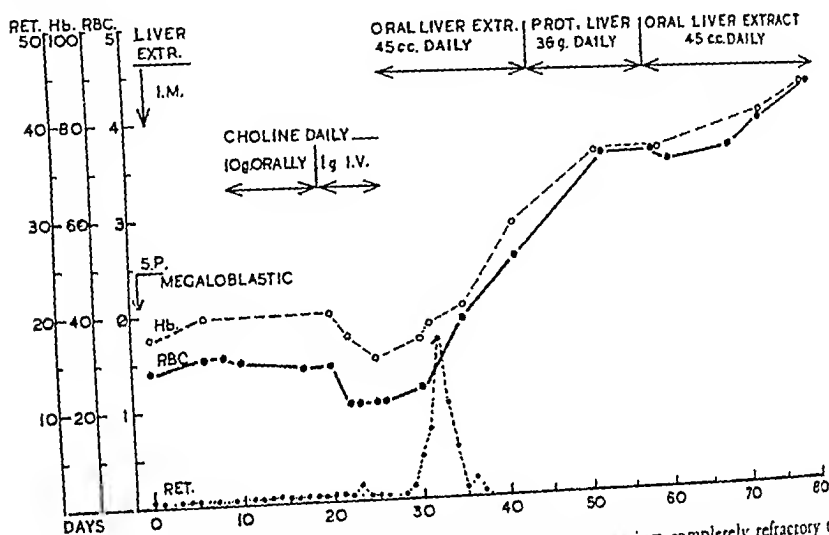


FIG. 4. A case of megaloblastic anemia of pregnancy and the puerperium completely refractory to numerous injections of liver extract. Choline given by the oral and intravenous routes was without effect, but subsequent oral liver therapy resulted in the restoration of a normal blood picture.

Laboratory findings: R.B.C., 1.4 M.; hemoglobin, 35 per cent; color index, 1.25; M.C.V., 115 cu. microns; W.B.C., 4,600 per cu. mm.; reticulocytes, less than 1 per cent. Blood film: macrocytic, hyperchromic. Sternal marrow: megaloblastic, hypercellular, no excess fat. Gastric analysis: free hydrochloric acid present in resting juice. Liver biopsy was not performed.

Treatment and progress: In view of the history, this case was diagnosed as a refractory megaloblastic anemia of pregnancy and puerperium, but to confirm the refractoriness to parenteral liver extract, an injection of anahaemin (4 cc.) was given. As shown in figure 4, this was without hemopoietic effect. The patient was then given choline—10 grams daily by mouth for ten days, followed by intravenous injections of 1 gram for a further period of seven days. This was followed by no reticulocyte response, while the red cell count fell. Oral liver extract was then given and resulted in a reticulocyte response of 17 per cent and a rapid rise in the red cell count from 1.05 M. to 3.64 M. in twenty-five days. Subsequently the count rose to 4.8 M. It remained at approximately this level during the following three months without further treatment.

*Case 5.* Mrs. M., aged 36, developed anemia during the latter half of her sixth pregnancy and was treated with iron. She was delivered of a healthy child but since the signs of anemia became more severe, a sternal puncture was performed seventeen days after delivery and an injection of liver extract (anahaemin, 4 cc.) was given on the same day. She came under our care six days later, on April 1, 1946.

Physical examination revealed no significant features other than those of anemia. Jaundice was not present and enlargement of liver, spleen or lymph glands was not demonstrated.

Laboratory findings: R.B.C., 1.56 M.; hemoglobin, 36 per cent; color index, 1.16; M.C.V., 109 cu. microns; reticulocytes, 4.5 per cent; W.B.C., 4,000 per cu. mm.; blood film, macrocytic. Sternal marrow: The films prepared on the occasion already mentioned were subsequently seen by us and showed megaloblastic erythropoiesis. Gastric analysis: free hydrochloric acid in resting juice.

Treatment and progress: A further injection of liver extract ("perhepar," 4 cc.) was followed by a delayed and suboptimal reticulocyte response, but the red cells showed a sustained rise, attaining a level of 3.2 M. by the twenty-fifth day, and then remained stationary at this level in the absence of further treatment for seventeen days. Choline chloride was then given intravenously in daily doses of 1 gram for ten days. A reticulocyte response of 3.5 per cent followed and a further rise in the red cell count occurred,

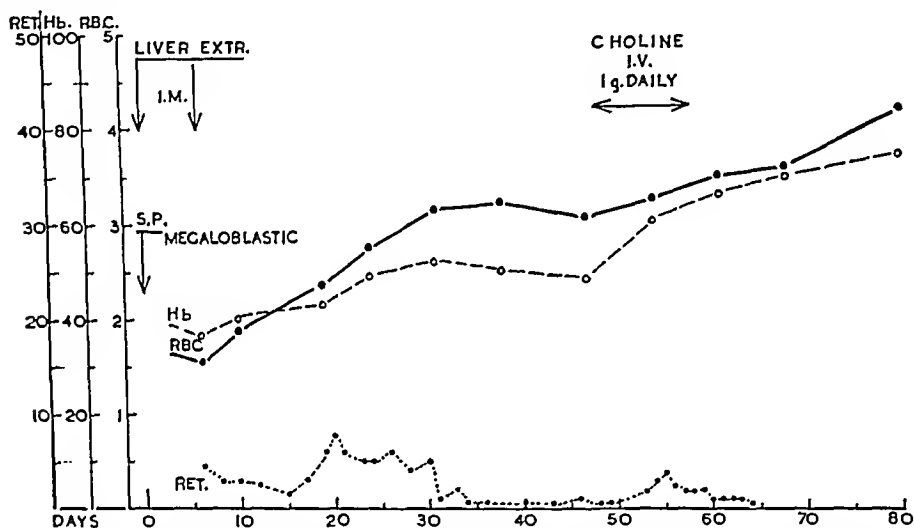


FIG. 5. A case of megaloblastic anemia of pregnancy and the puerperium. An injection of liver extract resulted in an erythropoietic response. Intravenous choline was followed by a slight reticulocyte response and a secondary rise in the red cell count.

reaching a level of 4.2 M, a month after commencing choline therapy (figure 5). No subsequent treatment was given and eventually the blood picture reached and remained at normality.

#### COMMENT ON CASES 4 AND 5.

Both these cases are clearly examples of megaloblastic anemia of pregnancy, as described by Davidson, Davis and Innes.<sup>5</sup> Case 4 was refractory to intensive parenteral liver therapy but subsequently responded to oral liver preparations. Choline, in the dosage employed, appeared to be completely lacking in effect in spite of numerous recent injections of liver extract. It would therefore seem in this case that the lacking erythropoietic factor, which parenteral liver therapy was unable to supply, was not choline or any agent which could be made available by choline.

Case 5 was an example of a common type of megaloblastic anemia of pregnancy which responds to parenteral liver therapy in the puerperium. As is well known, such cases frequently undergo spontaneous remission during the months following birth of the child. We therefore are of the opinion that it would be exceedingly

rash to conclude that the choline given in this case was necessarily responsible for the subsequently slow rise in the red cell count. The slight reticulocyte count, however, does appear to be suggestive that the choline may have exerted some erythropoietic effect. It should be noted that this apparent response to choline followed an earlier response to liver extracts.

#### C. MEGALOBlastic ANEMIA ASSOCIATED WITH THE SPRUE SYNDROME

*Case 6.* Miss S., aged 16, had suffered from celiac disease since infancy and came under our care in April 1946. She was an underdeveloped and poorly nourished girl, very small for her age. Many of the features of idiopathic steatorrhea were present, including osteoporosis, papillary atrophy of the tongue, slight clubbing of the fingers and the constant passage of bulky pale stools containing excess fat. The usual signs of anemia were present. There was no evidence of jaundice or of enlargement of the liver, spleen, or lymph glands.

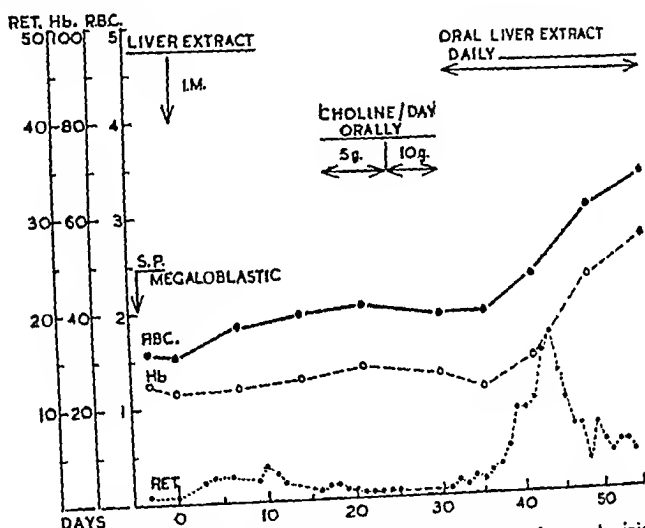


FIG. 6. A case of megaloblastic anemia associated with the sprue syndrome. An injection of liver extract followed by oral choline was without effect, but subsequent oral liver therapy resulted in a erythropoietic response.

Laboratory findings: R.B.C., 1.5 M., hemoglobin, 25 per cent; color index, 0.8; M.C.V., 100 cu. microns; M.C.H.C., 23 per cent; reticulocytes, 1 per cent; W.B.C., 8,500 per cu. mm. Blood films showed a "dimorphic" macrocytic, hypochromic picture. Sternal marrow: erythropoiesis was partly megaloblastic, many undoubted megaloblasts were present but the early megaloblasts were relatively small and normoblasts were relatively numerous. Gastric analysis: histamine-fast achlorhydria.

Treatment and progress: An injection of refined liver extract (anahaemin, 4 cc.) was followed by only a slight reticulocyte response and no sustained increase in the red cells (figure 6). Because of the patient's poor veins, repeated intravenous injections were not feasible and therefore it was decided to try the effect of choline by mouth; 105 grams of choline chloride given during 14 days resulted in no observable effect. Oral liver extract was then given and was followed by a brisk erythropoietic response, the reticulocytes rising to 17 per cent and the red cells from 2.0 to 3.4 M. within twenty-four days. After her discharge from the hospital she continued to take oral liver extract and when last seen the red cell count was 3.18 M. and her general condition improved.

*Case 7.* Miss P., aged 25, was apparently healthy until the age of 18 when she became anemic and was treated with iron and injections of liver extract. The injections were maintained, somewhat in

regularly, however, for seven years, when in spite of increased dosage her general condition deteriorated and the passage of loose pale stools was first noted. She was then investigated more fully in an outpatient clinic, when the diagnosis was established of idiopathic steatorrhea and of associated macrocytic anemia. At this time (November 1945) the salient laboratory findings were as follows: R.B.C., 2.33 M.; hemoglobin, 55 per cent; color index, 1.18; M.C.V., 107 cu. microns; M.C.H.C., 32 per cent; W.B.C., 8,900 per cu. mm.; plasma bilitubin, 0.5 mg. per cent; gastric analysis: free hydrochloric acid in fasting juice; fecal fat: 38 per cent of dried weight; glucose tolerance curve: flat; x-ray: skeletal osteoporosis.

The patient was subsequently kept under observation and on March 21, 1946, she developed a severe relapse necessitating her admission to hospital. Clinical examination revealed a short (5 feet) but relatively well nourished woman with no evident pathological signs other than those of severe anemia.

Laboratory findings: R.B.C., 1.2 M.; hemoglobin, 24 per cent; color index, 1.0; M.C.V., 101 cu. microns; M.C.H.C., 26 per cent; reticulocytes, less than 1 per cent; W.B.C., 5,000 per cu. mm.; sternal marrow, megaloblastic, hypercellular with no excess of fat.

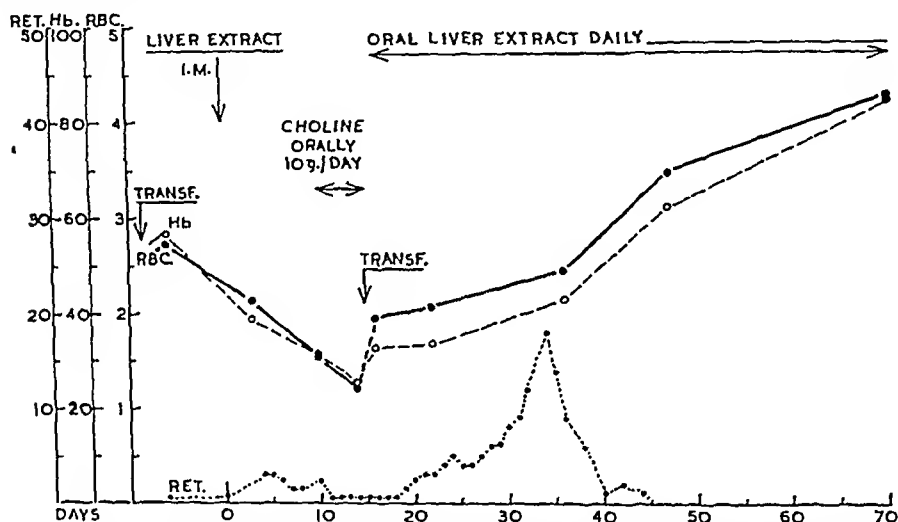


FIG. 7. A case of megaloblastic anemia associated with the sprue syndrome. No response occurred to an injection of liver extract followed by oral choline, but the administration of oral liver extract resulted in the restoration of a normal blood picture.

Treatment and progress: In view of her condition, she received a blood transfusion of 500 cc. of whole blood followed by 500 cc. of packed cells, which raised the red cell count to 2.7 M. Six days later an injection of liver extract (anahaemin, 4 cc.) was administered, but the red cell count fell to 1.54 M. in ten days. At this point choline chloride was given by mouth in daily doses of 10 grams, the oral route being chosen on account of the poor state of the patient's veins. Since no reticulocyte response occurred and the red cell count continued to fall, the choline was discontinued after six days and a further blood transfusion was given. Oral liver extract was then administered and, as seen in figure 7, resulted in a significant reticulocyte response and a progressive rise in the red cells to a level of 4.51 M., which has since been maintained for over a year, the patient continuing to take oral liver extract.

#### COMMENT ON CASES 6 AND 7

Although the investigation of these cases was not so complete as we would have desired, particularly in respect to fat absorption studies, there seems little reason to doubt that the megaloblastic anemia present was associated with an underlying defect in intestinal absorption. It is evident that in neither case could any erythro-

poietic effect be attributed to choline. It is unfortunate that in both cases the choline was given only by the oral route because, for reasons to be discussed later, it is probable that choline is less effective when administered by this route than when given intravenously.

It should be noted that in both cases the administration of choline was preceded by injections of liver extract, which were ineffective, and succeeded by oral liver therapy which was surprisingly efficacious hematopoietically.

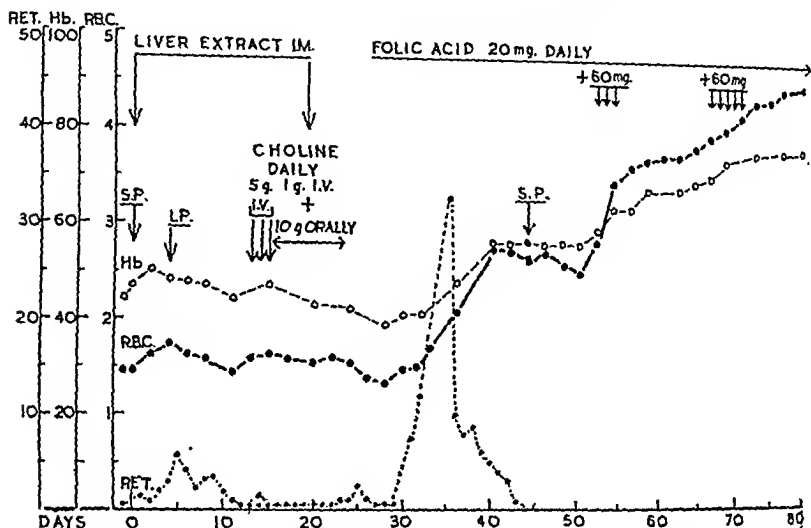


FIG. 8. A case of nutritional megaloblastic anemia which showed no significant response to an injection of liver extract or to choline given both intravenously and by mouth. The subsequent oral administration of folic acid resulted in a satisfactory erythropoietic response.

#### D. NUTRITIONAL MEGALOBlastic ANEMIA

*Case 8.* Mrs. A., aged 34, developed symptoms of anemia at the age of 28 and was subsequently treated intermittently with injections of liver extract. She came under our care on August 9, 1946. It is significant that she gave a history of an inadequate diet for some years, especially in respect of meat and vegetables. Clinical examination revealed, in addition to the usual manifestations of anemia, a dry brown pigmentation of the skin over the neck and backs of the hands. The tongue showed a slight degree of papillary atrophy and glazing of the tip. Her general nutritional state was poor. No jaundice was evident, nor was there enlargement of the liver, spleen, or lymph glands.

Laboratory findings: R.B.C., 1.48 M.; hemoglobin, 44 per cent; color index, 1.5; reticulocytes, less than 1 per cent; W.B.C., 5,400 per cu. mm. Sternal marrow: megaloblastic, hypercellular, no excess fat. Gastric analysis: free hydrochloric acid after injection of histamine. Liver biopsy: slight diminution in cellular glycogen and a slight increase in hemosiderin in both the littoral and liver cells, otherwise no abnormalities. Urine: no abnormalities.

Treatment and progress: An injection of liver extract (anahaemin, 4 cc.) was followed by a reticulocyte response of 6 per cent on the fifth day, but no significant rise in the red cell count. Choline chloride was administered over a period of ten days commencing on the thirteenth day after the injection of liver extract. As shown in figure 8, the choline was given intravenously 5 grams daily for three days, but on account of severe side reactions this was reduced to 1 gram for the remaining seven days during which time it was supplemented by 10 grams daily by mouth. A second injection of liver extract (anahaemin, 4 cc.) was given on the sixth day after commencing choline. This was given with the object

of providing an adequacy of the active principle in liver extract, and of thus testing the possibility that liver extract and choline might be active when given together, although inert when given separately. No such effect was noted, however, apart from a reticulocyte response of only 2 per cent, clearly of very doubtful significance, and the red cell count continued to fall. Three days after stopping the choline therapy, synthetic folic acid was given in daily doses of 20 mg. by mouth, which resulted in a reticulocyte response of 33 per cent on the eighth day and a brisk rise in the red cell count. A second sternal puncture now showed normoblastic erythropoiesis.

Continuation with folic acid therapy eventually resulted in the red cells rising to 4.75 M. This level has subsequently been maintained without further treatment.

#### COMMENT ON CASE 8

In view of the history and the clinical and laboratory findings, this case is regarded as an example of nutritional megaloblastic anemia refractory to treatment with refined liver extracts administered parenterally. It seems evident that choline chloride, in the doses employed, was without effect. It should be noted that although liver biopsy was performed in this patient, there was no evidence of gross fatty changes, nor were such changes seen in the sternal marrow films. The response to folic acid is of interest. It will be seen that the initial response was optimal, but the subsequent rise in the red cell count became retarded. Similar results with folic acid in the treatment of refractory megaloblastic anemias have been noted by Davidson and Girdwood.<sup>7</sup> In our case, however, it will be seen (fig. 8) that increasing the dose of folic acid was followed by further red cell regeneration.

#### E. REFRACTORY MEGALOBlastic ANEMIA OF UNCERTAIN ORIGIN

*Case 9.* Mrs. A. B., aged 51. A diagnosis of pernicious anemia had been made four years previously and injections of liver extract had apparently been successful for a time, but failure to continue treatment resulted in relapse. During the autumn of 1945, the patient was given numerous injections of liver extract by her doctor without improvement. She came under our care on January 29, 1946, in a state of moderately severe relapse despite numerous injections of liver extract given during the preceding weeks. The patient was an obese woman displaying the usual manifestations of anemia. No signs of jaundice were present and no enlargement was detected of the liver, spleen, or lymph glands. Signs of neurological disease were absent.

Laboratory findings: R.B.C., 1.88 M.; hemoglobin, 52 per cent; color index, 1.38; reticulocytes, less than 1 per cent; W.B.C., 3,400 per cu. mm. Sternal marrow: megaloblastic, hypercellular, no excess fat. Gastric analysis: histamine-fast achlorhydria. Urine: trace of urobilinogen.

Treatment and progress: An injection of liver extract (anahaemin, 4 cc.) was given but no reticulocyte response occurred within six days and the red cell count continued to fall. The oral administration of choline was then commenced and continued for three weeks, the dose being 3 grams daily. The following day the reticulocyte count was 4 per cent and rose to a maximum of 12 per cent on the ninth day after commencing choline and the fifteenth day after the injection of liver extract. It will be seen in figure 9 that the reticulocytes did not immediately return to a low level but continued at about 5 per cent during, and for a short time after, the first period of choline therapy. It will also be seen that the red cell count slowly rose during this period to a maximum of 2.39 M. A second sternal puncture performed at about this time, however, still showed frankly megaloblastic erythropoiesis. On the forty-second day after the first injection of liver extract and after the reticulocyte count had again fallen to less than 1 per cent, another injection of liver extract was given (anahaemin 2 cc.) and was followed by prompt reticulocyte response of 5 per cent but no rise in red cells. Thirteen days later, when the reticulocytes had again fallen to less than 1 per cent, the injection of liver extract was repeated (anahaemin, 2 cc.) and oral choline therapy recommenced in the same dosage as before. A similar reticulocyte response occurred, but again without a significant red cell increase. After two weeks of oral therapy the choline was given intravenously in daily doses of 1 gram and continued for ten days. This was accompanied by a reticulocyte response of 11 per cent and a significant rise in the red cell count which eventually reached 3.8 M. without

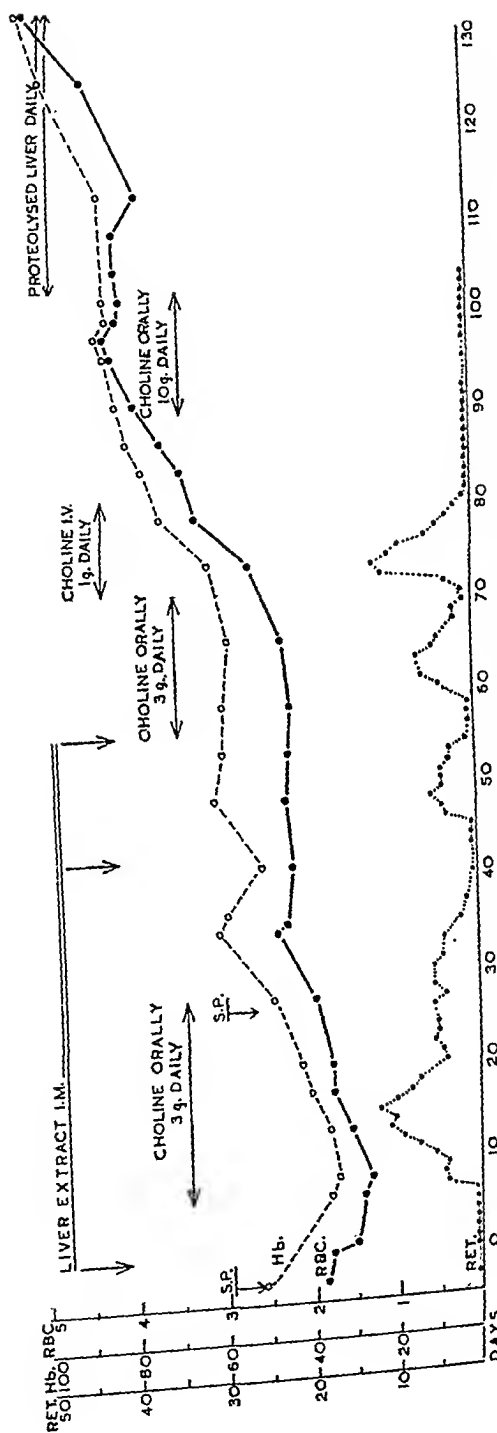


FIG. 9. A case of megaloblastic anemia resembling Addisonian pernicious anemia which had become refractory to numerous injections of liver extract. The chart illustrates the responses to oral and to intravenous choline. For details see text.

further treatment. A further course of oral choline, the dose now being 10 grams daily, was without effect on the reticulocytes. The red cells continued to rise to 4.15 M. but subsequently fell during the last period of therapy. Proteolyzed liver (36 grams daily by mouth) was then given and resulted in the eventual restoration of the red cell count to 5 M. The patient's general and hematological condition has since remained satisfactory on maintenance therapy with proteolysed liver.

*Case 10.* Mrs. S. B., aged 52, had been healthy until September 1946, when she developed symptoms of anemia. Following a diagnosis of pernicious anemia she received a course of injections of liver extract to which she failed to respond. She was then seen by one of us on December 8, 1946. The usual symptoms and signs of anemia were present and a mild degree of jaundice was noted, but there was no demonstrable enlargement of the liver, spleen, or lymph glands, or other significant pathological features.

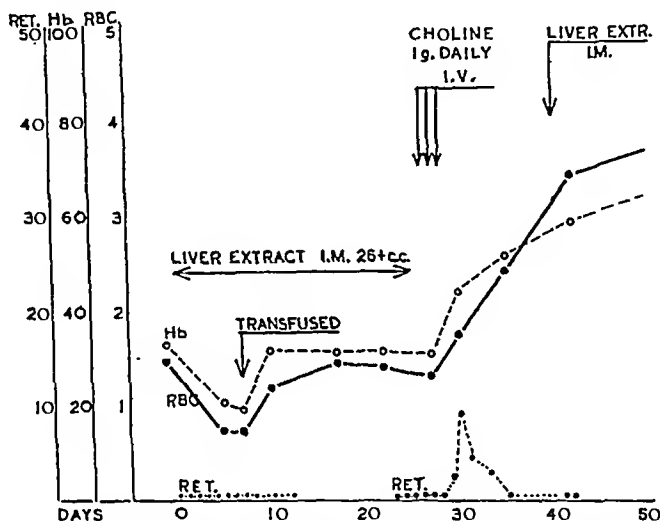


FIG 10. A case of megaloblastic anemia of uncertain etiology refractory to numerous injections of liver extract. The intravenous injection of choline over a period of only three days was followed by a significant response. The patient subsequently responded to parenteral liver therapy.

Laboratory findings: R.B.C., 1.4 M.; hemoglobin, 31 per cent; color index, 1.1; reticulocytes, less than 1 per cent; white cells, 4,000 per cu. mm. Sternal marrow: megaloblastic, hypercellular, no excess fat. Gastric analysis: histamine-fast achlorhydria.

Treatment and progress: The patient had already received frequent injections of liver extract totaling at least 26 cc. during the previous three weeks (see fig. 10). This had resulted in no improvement in her red cell count; reticulocyte counts, however, had not been recorded throughout the whole of this period. Choline was given intravenously in daily doses of 1 gram for three days and was followed by a reticulocyte response of 9 per cent on the fifth day, and a rise in the red cell count from 1.3 M. to 3.4 M. on the seventeenth day. The patient was subsequently treated with injections of liver extract (anahaemin) with consequent restoration and maintenance of a normal blood picture.

#### COMMENT ON CASES 9 AND 10

The etiological status of case 9 is obscure. A diagnosis of Addisonian pernicious anemia would be justified were it not for the inadequate response to parenteral liver therapy. This case corresponds to a type of idiopathic refractory megaloblastic anemia previously described by Davidson, Davis, and Innes,<sup>3</sup> and Davis and David-

son<sup>6</sup> and in many respects it is similar to that of Moosnick, Schleicher, and Peterson,<sup>1</sup> although jaundice and gross disturbances of fat metabolism were features of their case. No liver biopsies were performed in our case, but the sternal marrow smears provided no evidence of gross fatty changes as illustrated by these authors. It is unfortunate that the first period of oral choline therapy was commenced so soon after the injection of liver extract, since it is impossible to exclude the effect of the latter in producing the subsequent reticulocyte response. In view, however, of the absence of any reticulocyte response within seven days of the injection of liver extract, it would seem that the choline probably played at least some part in the production of the reticulocyte and red cell response. It must be remembered, furthermore, that numerous injections of liver extract had been given during several weeks immediately preceding the patient's admission to hospital. During the next phase of the experiment two injections of liver extract were given, the second of which was followed by a further course of oral choline. The response to each of these was identical, namely, a slight reticulocyte response but no increase in the red cells. The subsequent period of intravenous choline therapy, without further liver extract was, however, followed by another and greater reticulocyte response and a significant rise in the red cell count. It may be concluded therefore from this case that the choline chloride administered intravenously exerted a significant erythropoietic effect, and that choline by mouth probably produced some effect.

In case 10 the response to choline seems unequivocal. It will be noted that this patient proved refractory to numerous injections of liver extract given shortly before the administration of choline. In view of the patient's subsequent response to further injections of liver extract, it would appear that the earlier refractoriness to parenteral liver extract was a temporary phase. Whether choline played a part in overcoming this refractory phase is, of course, conjectural. We feel that it is particularly unfortunate in this case that no liver biopsy was done before the institution of choline therapy. It is perhaps significant, however, that the sternal marrow material showed no evidence of the fatty changes reported by Moosnick et al.<sup>1</sup>

## DISCUSSION

### ANALYSIS OF RESULTS

The following conclusions are suggested by our observations.

1. Choline by itself is incapable of rectifying megaloblastic erythropoiesis or of exerting a significant erythropoietic effect. This conclusion is based upon case 1, an example of untreated Addisonian pernicious anemia which showed no response to choline but responded satisfactorily to subsequent injections of liver extract. There are, of course, no theoretical grounds for believing choline to possess intrinsic hematinic activity. On the contrary, choline has been claimed to depress erythropoiesis by its vasodilator action in increasing the oxygenation of the bone marrow (Davis<sup>8</sup>).

2. Choline appears to be capable of exerting a "boosting" effect in cases of megaloblastic anemia in which a response to parenteral liver therapy has already occurred.

This conclusion is based on cases 2, 3, and 5, representing two examples of Addi-

sonian pernicious anemia and one of megaloblastic anemia of pregnancy respectively. It will be recalled that these cases all responded to one or more injections of liver extract and that the administration of choline after the erythrocyte count had ceased to rise, or had actually begun to fall, was followed by a secondary rise in the red cells. Admittedly, a reticulocyte response to choline was noted only in case 5, and in this case the need for caution in its assessment has already been stressed. Nevertheless, in view of the uniform pattern of the erythrocyte response displayed by each of these cases, we believe that it is justifiable to attribute the effect of choline.

3. The effect of choline in megaloblastic anemias refractory to parenteral liver therapy is variable and appears to depend upon the type of case and its underlying etiology. Cases falling under this heading which are included in this study may be divided into two broad groups according to their erythropoietic response to choline.

The first group, consisting of 4 cases all of which showed no response to choline despite intensive parenteral liver therapy immediately preceding its administration, comprise examples of well recognized conditions, namely, anemias associated with pregnancy (case 4), with the sprue syndrome (cases 6 and 7), and with nutritional deficiency (case 8). It is now generally recognised that a considerable proportion of such anemias may show varying degrees of refractoriness to parenteral liver therapy while responding promptly to the administration of liver products by mouth, (Davis and Davidson,<sup>6</sup> Davis,<sup>9</sup> Fullerton,<sup>10</sup> Watson and Castle<sup>11</sup>). Possible explanations for this phenomenon have been discussed by Davis and Davidson<sup>6</sup> and Watson and Castle,<sup>11</sup> although recent work on folic acid may lead to a reorientation of the problem (see Davis<sup>12</sup>). Whatever may be the explanation for the efficacy of oral liver therapy in such cases, our present studies provide evidence that the lack of response to refined liver extracts administered parenterally cannot be corrected by choline. In this connection it is significant that the choline content of proteolyzed liver is approximately 36 mg. per 100 grams (Riding<sup>13</sup>) which would amount to a daily dosage of only 12 mg. in the dose of proteolyzed liver usually employed. It should be noted that in our two examples of megaloblastic anemia associated with defective intestinal absorption (cases 6 and 7) the choline was given only by the oral route although in large doses. Since it is probable that this method of administration is less effective than the intravenous route, it may be objected that the negative response of these two cases is inconclusive. On the other hand, if choline were capable of exerting an erythropoietic effect in such cases by promoting intestinal absorption (Frazer<sup>2</sup>) it would seem probable that the oral route would be effective.

The second group consists of the two cases, 9 and 10, classified as refractory megaloblastic anemia of unknown origin. Both these cases would have been regarded as classical examples of Addisonian pernicious anemia had it not been for their ineffectual response to potent parenteral liver extracts. Case 9, in fact, had previously responded to such treatment but subsequently became refractory. In both cases choline, given a short interval after intensive parenteral liver therapy, was followed by a significant erythropoietic response, and in case 10 the patient subse-

quently became amenable to injections of liver extracts. It seems probable, therefore, that in these two cases the nature of the refractoriness to parenteral liver therapy may differ fundamentally from that of the first group discussed above. It is perhaps significant that these cases resemble in many respects the case described by Moosnick et al.,<sup>1</sup> although in their case jaundice and severe hepatitis were present.

#### THE MODE OF ACTION OF CHOLINE

Moosnick et al.<sup>1</sup> suggest that the refractoriness of their patient to parenteral liver therapy was due to an underlying choline deficiency which resulted in severe hepatic disease and disordered fat metabolism, thus rendering the liver incapable of utilizing or of elaborating the active principle supplied by the liver extracts. Although it is well known that the liver is concerned with the storage of the antianemic principle and occasional cases of megaloblastic anemia have been attributed to hepatic disease, so far as we are aware there is no conclusive evidence that the therapeutic activity of liver extracts in pernicious anemia is dependent upon hepatic function. In this connection, perhaps it is not irrelevant to refer to recent work on folic acid, which, if confirmed, may provide an attractive, but at present entirely speculative, theory of the role of the liver in erythropoiesis.

The experiments of Welch et al.<sup>14</sup> suggest that the essential defect in pernicious anemia is an inability on the part of the organism to liberate free folic acid from inactive conjugates assimilated from the food, and that the hematopoietic activity of liver extracts is concerned with the restoration of this function. Although the site of this action suggested for the active principle of liver extracts is unknown, the possibility that it may be in the liver merits consideration. For if this were confirmed it would provide support for the view that the lack of response to parenteral liver extracts displayed by certain types of megaloblastic anemias is due to hepatic dysfunction. Moreover, the action of choline in overcoming the refractoriness of such cases would be comprehensible in view of the recognized influence of this substance upon the metabolism of liver cells.

The satisfactory response of cases of this type to oral liver therapy may possibly be explained by the assumption that the oral route results in the liberation of free folic acid from conjugates within the alimentary tract from which it is absorbed and utilized by the bone marrow independently of hepatic function. It must be noted, however, that Doan<sup>15</sup> refers to a case of megaloblastic anemia complicated by hepatic cirrhosis which was refractory to folic acid.

The apparent "boosting" effect of choline in those cases in which a response to liver extracts has already occurred may conceivably be due to the action of choline in stimulating relatively healthy liver cells to further activity which, in the presence of a residuum of the active principle, enhances the production of folic acid.

Acceptance of this theory of hepatic dysfunction as the cause for refractoriness to parenteral liver does not, in our view, necessarily imply that the disorder is caused by an inadequate intake of choline. In the presence of established disease, the choline requirements of the liver may well rise considerably above the normal. The therapeutic effect of the administration of choline does not therefore justify the assumption that nutritional deficiency is necessarily concerned in etiology.

Although plausible, this theory of hepatic dysfunction as a cause of refractory megaloblastic anemia is obviously based upon very slender evidence, and the objections to it are manifest. It is well known that the functional reserve of the liver is considerable and that adequate function is compatible with extensive pathological changes. In none of our cases were there manifestations of hepatic dysfunction other than slight icterus and urobilinogenuria, although it must be admitted that liver biopsies and comprehensive liver function tests were not performed in cases 9 and 10. Moreover, many cases of pernicious anemia show marked jaundice, and presumably in long-standing untreated cases fatty and other changes are frequently present in the liver, yet lack of response to parenteral liver therapy is exceedingly rare.

Apart from its influence upon the liver, other effects of choline must be considered in seeking an explanation of its erythropoietic activity. Thus, it is possible that this is due to the influence of choline upon the metabolic activities of other tissues such as the bone marrow. Alternatively, the erythropoietic action of choline may result not from its lipotropic or other influence upon metabolism but simply from its vasodilator effect. Although choline has been shown to depress erythropoiesis in normal animals (Davis<sup>8</sup>), it is conceivable that it may exert a reverse effect under the abnormal conditions obtaining in megaloblastic anemias.

A point already referred to, but one deserving further emphasis, is that the demonstration of an erythropoietic response to choline does not necessarily imply that the patient was suffering from a nutritional choline deficiency. The established pharmacological effects of choline and related substances are almost certainly the result of its action, in relatively high concentration, upon susceptible cells and not due to the restoration of a normal physiological level. This question is of some practical importance, because if the response to choline is due to the correction of a deficiency, it follows that refractoriness to parenteral liver may be due to faulty nutrition. This seems improbable, however, since our patients in whom choline was effective (2, 3, 5, 9, and 10) presented no unusual subjective or objective evidence of nutritional deficiency, while the patients who were believed to be in an unsatisfactory nutritional state (4, 6, 7, and 8) showed no response to choline. Although this reasoning is not conclusive, it does suggest that if choline deficiency plays a significant part in the etiology of the type of case under discussion, it is probably conditioned by an intrinsic defect, rather than the result of inadequate intake.

#### DOSAGE AND ROUTE OF ADMINISTRATION OF CHOLINE

Our data are too scanty to justify dogmatism regarding the optimal dosage and method of administration of choline chloride. Nevertheless, consideration of case 9 suggests that while choline given by mouth is not without some erythropoietic effect, it is probably more efficacious when administered intravenously, despite a considerable reduction in dosage. The reason for this is difficult to explain, since numerous animal experiments have shown that choline by mouth exerts an effective lipotropic action. Presumably the intravenous injection, even of relatively small doses, results in a higher, although more transient, blood concentration of choline than the administration of larger doses by mouth. This perhaps suggests that the

erythropoietic effect of choline depends upon a positive action rather than upon the rectification of a deficiency. On the other hand the possibility must be considered that interference with absorption of choline from the alimentary tract may be responsible for the greater efficacy of its intravenous administration.

The difficulties of high dosage by the intravenous route have already been discussed. Daily intravenous injections of 1 gram of choline chloride, however, were usually without unpleasant side effects, and since this dosage was effective in several of our cases, as well as in that of Moosnick et al.<sup>1</sup> it seems probable that, in cases likely to respond to choline, this dose may be adequate. The possibility must be admitted that a higher dosage may be desirable to ensure the optimal erythropoietic effect,<sup>16</sup> although it should be noted that some of our patients who did not respond to choline received it in doses of 10 grams daily, both orally and intravenously.

#### THE CLINICAL VALUE OF CHOLINE AS A HEMATINIC

Our results do not suggest that choline will be of much practical value in the treatment of megaloblastic anemias, because only a small proportion of cases refractory to parenteral liver therapy are likely to respond to choline. On the other hand, there are good reasons for believing that the great majority, if not all, of such cases can be treated effectively with oral liver preparations. It is possible, however, that choline may be of value in exceptional cases complicated by severe hepatic disease, such as that of Moosnick et al.,<sup>1</sup> but this point cannot be decided until more information is available concerning the effect of oral liver or folic acid therapy in this type of case.

Despite the limited applications of choline in therapeutics, the apparent erythropoietic effect of this substance seems to be of sufficient academic interest to warrant further study, which might well be extended to embrace other substances having similar pharmacological actions.

#### SUMMARY AND CONCLUSIONS

1. The effect of the administration of choline chloride has been observed in 10 cases of megaloblastic anemia of various types.

2. Choline was without effect in a case of untreated Addisonian pernicious anemia which subsequently responded to parenteral liver therapy.

3. Choline was also without effect in a case of nutritional megaloblastic anemia, in a case of megaloblastic anemia of pregnancy, and in two cases of megaloblastic anemia associated with the sprue syndrome. All these cases had proved refractory to injections of potent liver extract before the choline was given, and all responded to subsequent oral liver or folic acid therapy.

4. A significant erythropoietic response to choline occurred in two cases resembling Addisonian pernicious anemia which were refractory to parenteral liver extracts.

Secondary responses followed the administration of choline in two other cases of Addisonian pernicious anemia and in a case of megaloblastic anemia of pregnancy, all of which had already responded to injections of liver extract.

5. The significance of these observations is discussed. It is concluded that choline possesses no direct erythropoietic activity, but that under certain circumstances it may potentiate the effect of liver extracts.

It is suggested that refractory megaloblastic anemias may be divided into two groups. In one, represented by well known syndromes associated with defective absorption or pregnancy, the lack of response to parenteral liver extracts is not corrected by choline. In the other, represented by two cases simulating Addisonian pernicious anemia, choline is effective in overcoming, partially or completely, the refractoriness to parenteral liver therapy. Consideration is given to the view that the refractoriness of this group results from hepatic dysfunction.

6. The most satisfactory method of administering choline probably consists of intravenous injections in daily doses of 1 gram. Larger doses given intravenously are frequently accompanied by unpleasant side effects, while oral administration appears to be relatively less effective.

7. It seems unlikely that choline will be of practical value in the treatment of refractory megaloblastic anemias, for which oral liver preparations provide the most certain and effective treatment. It is possible, however, that choline may be of use in cases complicated by severe hepatic disease.

Acknowledgment: We wish to thank Dr. L. D. W. Scott for permission to include his patient (case 10) in our series.

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## SUCCESSFUL TREATMENT OF LIVER-REFRACTORY ANEMIA WITH SYNTHETIC LACTOBACILLUS CASEI FACTOR

By JAN WALDENSTRÖM, M.D.

THE TREATMENT of pernicious anemia that is refractory to liver has been one of the most difficult problems in hematology. In many of these cases the result of treatment with liver extracts is excellent for some time and everything seems well. Later the patient responds less and less to antianemic treatment and the final outcome is usually fatal. Before discussing the favorable results of treatment with folic acid in such conditions, it may seem appropriate to treat some questions of nomenclature briefly. For example, it is evident that the name "folic acid" is not a synonym for the synthetic lactobacillus casei factor. For the sake of brevity, however, it is used here in this meaning.

In 1935 and 1936, Israëls and Wilkinson introduced the expression *achrestic anemia* to characterize cases showing a pernicious anemia-like blood picture but having a normal gastric acidity, no disturbance of the gastrointestinal tract, no involvement of the central nervous system, no pyrexia or evidence of hemolysis, and a lack of or a poor response to antianemic treatment. The bone marrow was described as megaloblastic.

Since the first delimitation of this picture, a few cases have been published by various authors but not by as many as might be expected from the comparatively large series of cases described by the original authors. On the other hand, their experience with uncomplicated pernicious anemia is unusually large. The incidence of achrestic anemia in Manchester, England, was stated to be 1 per cent of 600 cases of typical pernicious anemia. It is, however, hard to say anything definite about the real frequency of this disease in other populations. According to our opinion it would seem to be too early to decide whether these cases constitute a homogenous group or are instances of different pathological mechanisms (cf., e.g., Davis and Davidson). The term *achrestic anemia* has not been universally adopted. In the German literature Schulten (1939), in his textbook on diseases of the blood, does not mention the term and Heilmeyer (1942) regards the entity as not yet well founded. He seems to believe that these cases should be regarded as instances of aplastic anemia. In Switzerland, Rohr (1940) does not treat the question of achrestic anemia in his monograph on the bone marrow. In American literature there is some discussion about the real connection of these cases with pernicious anemia. Wintrobe (1942), for instance, takes up this problem in his *Clinical Hematology* and thinks that the cases show more resemblance to the cases of progressive hypocythemia described by Thompson. The latter are discussed under the title aplastic anemia. It is obvious that this is true of a large number of cases that are regarded as liver-refractory pernicious anemia.

In their second paper, published in 1940, Israëls and Wilkinson described further cases of achrestic anemia. The incidence was still about 1 per cent in their total

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material of 1100 patients with pernicious anemia. The 6 new patients were divided into two groups, 3 being regarded as typically achrestic. The others resembled somewhat pernicious anemia in pregnancy. All the patients had a typically megaloblastic marrow, as shown convincingly in a number of photomicrographs. It was found that the young patients reacted somewhat better on liver treatment.

Case reports of achrestic anemia have also been published by Abrahamson and Thompson (1937), Wauchope and Leslie-Smith (1938), Hynes (1939), and, probably, by Dameshek and Valentine (1937).

A very interesting report on 6 cases of liver refractory anemias has recently been made by Davis and Davidson (1944). Three cases in this paper are regarded as achrestic anemia by the authors. The cases were treated with proteolyzed liver after it had been shown that they were refractory to potent liver extracts. The reactions were favorable. The authors are of the opinion that proteolyzed liver perorally supplies some hematopoietic factor lacking in fractionated liver extracts. All the patients had previously received large doses of potent liver extracts, and the authors think it likely that they needed an additional factor for normal blood formation. The question of several factors will be discussed later in this paper.

Since 1937, when we had the opportunity of following the development of a typical instance of achrestic anemia in Upsala, we have been convinced that this represents a clear-cut syndrome. I shall give the case history briefly, as it shows some interesting features.

*Case 1.* A woman, born in 1891. Father died of gastric carcinoma. Mother probably also had carcinoma. Since 1925 dyspeptic troubles with vomiting, anorexia, and loss of weight. Admitted to the Surgical Clinic in 1926. No achlorhydria, no melena. Later, increasing number of stools, rather loose. Admitted to the Medical Clinic in 1928. Very thin. Tongue normal. Hemoglobin 57 per cent; R.B.C., 4.2 M., W.B.C. 9,000; platelets, 350,000. Some normoblasts in the blood smear. No achlorhydria. Treated with arsenic and iron. In November 1929, suddenly blind in right eye; pregnant. Admitted to the Obstetric Clinic. Anemia. R.B.C., 2.16 M.; thrombosis central artery of retina; later thrombosis in both legs. Postpartum increasingly anemic with excessively low blood values. R.B.C., 0.68 M.; 123 normoblasts/100 W.B.C., and large numbers of megalocytes. Treated with blood transfusions, iron, liver extracts, liver diet and arsenic. The blood values slowly improved. In 1930, R.B.C. 4.0 M. Readmitted to the Medical Clinic in February 1934 for *diarrhea*. Three to four loose, foul smelling movements daily. Quite emaciated. Very pale. Blood pressure 95/50. Hemoglobin, 30 per cent; R.B.C., 1.4 M.; W.B.C., 4,400. Normoblasts 31/100 W.B.C. Treatment with campolon gave rather good results, with a hemoglobin of 80 per cent. R.B.C., however, only increased to 3.5 M. and later declined in spite of continued treatment. In July 1935, back pain, loss of weight, glossitis. Later, fever with some coughing. Admitted in January 1936. Tongue smooth; neurologically negative; blood pressure 130/95; hemoglobin, 80 per cent; R.B.C., 2.43 M.; W.B.C., 4,300; neutrophils, 37 per cent; lymphocytes 58 per cent; monocytes 5 per cent. Normoblasts 194/100 W.B.C., i.e., a very considerable leucopenia. Large numbers of *Howell-Jolly bodies* (54/500 R.B.C.). Platelets, 182,000; reticulocytes 4 per cent. *Some hydrochloric acid after caffeine*. The spleen could not be seen on roentgenograms. The patient was treated with campolon. Readmitted in March 1937. She had had periods of diarrhea with fever. Still severe hyperchromic anemia that did not react to campolon. X-rays of the spine showed *considerable osteoporosis* with biconcave vertebrae. The patient was treated with large amounts of campolon injections and hepamult and hepaforte perorally but these gave no lasting effect. She was also given transfusions but her general condition deteriorated. In spite of the intense antianemic treatment the bone marrow contained *typical megaloblasts*. She ran a slight fever and had an increased sedimentation rate. Later a lump was palpated in the abdomen to the left but the roentgenogram showed no signs of gastric carcinoma. The later development was dominated by an acute right-sided otitis that never subsided. The patient was transferred to the Otiatric

Department, where she later died. It was found that the palpable masses in the abdomen were caused by enlarged retroperitoneal glands which were diagnosed by the pathologists as Hodgkin's disease. There was found a marked atrophy of the spleen, confirming the clinical diagnosis of *hyposplenism*.

#### COMMENTS

The syndrome represented by this patient showed some remarkable features. She had a hyperchromic, megaloblastic anemia that responded rather well to treatment with liver extracts for several years. The peculiar thing was that her gastric juice contained free hydrochloric acid. A large number of nucleated red cells in the blood and erythrocytes containing Howell-Jolly bodies was regarded as evidence of splenic atrophy. This diagnosis was confirmed at autopsy. The clinical diagnosis in this case was nontropical sprue of the type associated with atrophy of the spleen, but it could just as well have been achrestic anemia. It must be regretted that analyses of fecal fat were not performed, nor was a glucose tolerance test made. The combination of chronic diarrhea with osteoporosis, liver refractory megaloblastic anemia, and atrophy of the spleen in the absence of achlorhydria places the patient in the group of nontropical sprue without any doubt (see also case 5).

The combination of *idiopathic steatorrhea* and *atrophy of the spleen* has been known for only a comparatively short time. Dünner, Hirschfeld, and Gerald and Holz and Rohr discussed the possibility of atrophy of the spleen in these conditions but were unable to prove it at autopsy. The first anatomical confirmation was given by Engel, who published a case of nontropical sprue from Sweden in 1931. The patient died in 1934 and an atrophic spleen weighing only 12 Gm. was found. The histologic picture showed severe sclerosis with only small remnants of the pulp in the form of follicles. No signs of any specific disorder were found. Re-examination of blood smears by Engel in 1939, when the case was again published, showed that 8 per cent of the erythrocytes contained Jolly bodies. At the discussion following Engel's paper, Strandell gave a short account of a woman suffering from liver-refractory hyperchromic anemia with leucopenia and thrombocytopenia but without achlorhydria. Seven per cent of the erythrocytes showed Jolly bodies. The spleen weighed only 60 Gm. at autopsy. Another case history was briefly related by G. A. Johansson. Hyperchromic anemia, some leucocytosis, very marked thrombocytosis, and large numbers of Jolly bodies were regarded as possibly indicating atrophy of the spleen. A glucose tolerance test showed a very slight increase after a dose of 1 Gm./Kg. body weight. It is thus possible that this condition is not extremely unusual. Engel has shown that a number of previously published cases with atrophy of the spleen that had signs of chronic enteritis may well be interpreted as nontropical sprue. The biological importance of these interesting findings will not be discussed here. From a diagnostic point of view it is important to know that atrophy of the spleen may be one of the symptoms that should be looked for in cases of liver-refractory macrocytic anemia.

I have previously suggested that nontropical sprue may be the diagnosis in some instances of achrestic anemia. Since this case was seen, we have not had another instance of achrestic anemia in ten years. During this time about 200 new cases of uncomplicated pernicious anemia from the province of Upsala have been diagnosed

in the Medical Clinic. The incidence of liver-refractory pernicious anemia has therefore been 2/200, a number that fits in quite well with Wilkinson's 1 per cent in Manchester. In our country this may therefore be the approximate frequency of the condition. The other cases published in this paper come from other parts of Sweden and should therefore not be counted in this connection. Our case 2 of liver-refractory anemia had the following history.

*Case 2.* W. B. Woman born in 1890.\* Hereditarily nothing of importance. No maladies since 1908, when she had a chronic nontuberculous mastitis. In 1929, chronic eczema. In 1932, hypochromic anemia treated with iron. Regarding the later development of blood values, see fig. 1. Admitted to the Medical Clinic in 1933 with signs of pernicious anemia. Tongue completely smooth; spleen enlarged. W.B.C., 3,100; platelets 112,000. After two months of treatment with campolon and iron, the patient was dismissed with normal blood values. Readmitted in November 1939 for symptoms relating to the mucous membranes with glossitis, dysphagia and perlèche. Otherwise in good condition. Unusually large fissures in the corners of the mouth. Tongue absolutely smooth, as though polished. Also signs of general stomatitis. Skin somewhat diffusely pigmented with numerous dark patches. Heart: systolic murmur over the whole precordium. Liver and spleen not palpable. No urobilinuria. Blood pressure, 120/75. Icterus index, 3. It was regarded as probable that the patient suffered from iron deficiency and she was treated with large doses of iron without any definite improvement of the oral symptoms. In 1940 she was again treated with large doses of iron without any definite effect. Later dry yeast for one period, riboflavine 6 mg. daily for a considerable time, and pyridoxine in injections during different periods—all this without lasting effect on the fissures or the tongue papillae. She was therefore readmitted to the clinic in June 1943. Tongue still completely smooth. She was now treated with "becozym" but without any real improvement. In March 1945 she was rather ill with anorexia, vomiting, and diarrhea. Severe stomatitis. Gait unsteady with paresthesias in hands and feet. Blood values steadily decreasing (fig. 1) in spite of continued treatment. Sternal puncture: megaloblastic marrow. No Jolly bodies in smears of the periphery, blood. Admitted to the Medical Clinic on October 26, 1945. Tongue completely smooth with aphthoid ulcers. Spleen somewhat enlarged, easily palpable. Blood pressure rather low (95/65). For blood values see figure 2. Bone marrow as before. The patient was treated with injections of liver extracts and later with transfusions (fig. 2). The result of the liver treatment as regards hemoglobin and R.B.C. was negative but there was an increase in platelets from 38,000 to 143,000. Roentgenogram of the esophagus showed no varices or other pathological changes. Sternal puncture: scattered megaloblasts. Takata reaction negative. Quantitative blood bilirubin, 0.7 mg. per cent. Icterus index, 2. Red cell fragility, 0.44-0.26 per cent NaCl. Roentgenogram of the thoracic spine showed no osteoporosis. Serum calcium, 8.6 mg. per cent. No occult blood in the stools. An analysis of fecal fat in the Department of Physiological Chemistry (Professor G. Blix), showed a content of 18.5 per cent with 49 per cent free fatty acids, 41.5 per cent neutral fat and 9.5 soaps. Fractional test meal: no hydrochloric acid after caffeine. After 0.5 mg. histamine: free acid 14, total acid 28. The patient later showed a rise in temperature (maximum 39.7° C) with marked decrease of W.B.C. (minimum of 1,100). She was treated with penicillin, 12,000 units  $\times$  8 for 6 days. The high temperature slowly subsided but remained subfebrile and the sedimentation rate was high, 70-80 mm./hr. The patient was given several transfusions but her status remained very unsatisfactory.

When she was discharged on December 17, 1945, it was noted in the case history that the possibility of a splenic anemia must be taken into account. Readmitted on January 23, 1946. Very tired. No glossitis. Insomnia, coughing, tachycardia, swelling of the legs, slight diarrhea, no paresthesias, quite emaciated. Weight, 42 Kg; tongue absolutely smooth and glistening; fissures now healed. Blood pressure,

\* The case history has previously been related by Agren at the meeting of the Swedish Physiological Society in Stockholm, March 18, 1946 (see Sv. Läkarsidn., April 12, 1946) and by Waldenström at the meeting of the New York Academy of Science on April 20, 1946. Some further results of folic acid treatment in liver refractory anemia were discussed by Waldenström at the general meeting of the Nordic Society for Internal Medicine in Gothenburg, June 28, 1946. At the same meeting Espersen and Jørgensen reported on a case of liver refractory pernicious anemia successfully treated with folic acid.

12/5/75. Liver palpable 6 cm. below the costal margin; spleen 2 finger-breadths below the costal margin. Neurologically nothing objective. For blood values, see figure 3. N.P.N., 24 mg. per cent. Icterus index, 4. Takata negative. Blood proteins, 5.1 per cent; albumin, 2.5; globulin, 2.6; fibrinogen, 0.3 per cent.

At this time, Dr. Gunnar Ågren of the Department of Physiological Chemistry obtained some samples of folic acid through the courtesy of A. B. Ferrnsan. Because of the very marked leucopenia, it was decided to try the preparation on this patient. As regards the effect, see figure 3.

The patient was dismissed in a rather satisfactory condition after a total dose of 2,300 mg. of folic acid. No further treatment was given and the patient was followed in the Out-Patient's Department. Her general condition of health has been good and she has been able to do her usual household work.

Readmitted in August 1946 as the status of her tongue was not satisfactory. There were found a few very low papillae on the back of the tongue and several small ulcers. Big fissures in the corners of the mouth. Liver and spleen still increased in size. As the serum iron value was persistently low, the patient was given iron perorally and as intravenous injections in a dosage of 10 mg. of iron once daily. This treatment did not materially change the condition of her mucous membranes. She was discharged on iron therapy but later studies of the tongue status showed no improvement and the serum iron values remained low. Her last blood status was taken 10 months after her folic acid treatment, except a period of 7 days in September when she was given a daily dose of 15 mg. against her glossitis. It was Hb., 91 per cent; R.B.C., 4.4 M.; W.B.C., 3,500; and serum iron 44.7 per cent.

#### COMMENTS

The anemic condition began as hypochromic anemia in 1932. She is thus an instance of the transition from hypochromic to hyperchromic "pernicious" anemia. This condition is very common in Upsala, having been seen in 28 cases of a total of 137 female cases with pernicious anemia from the province (Waldenström, 1944). When she came back in 1933 she was treated with both liver extract and iron and it seems certain that it was the liver that brought about the improvement, as the anemia was definitely macrocytic. It must be regretted that reticulocyte counts were not made nor were sternal punctures performed at this early stage of the disease. In the meantime her blood values were kept very satisfactorily on continuous treatment with liver extracts with intervals of about six to 8 weeks. It should be noted, however, that both in 1939 and in 1941 an occasional leucocyte count showed strikingly low values. In the year 1944 the values for the red cells were normal as before, but in 1945 there was a marked decline and in the autumn of 1945 the patient became desperately ill in spite of large doses of potent liver extracts. This is shown in figures 1 and 2. From these charts it may be noted that heptomin in large doses gave a slight drop in serum iron and a very feeble reticulocyte peak but no improvement of either the red or the white cell counts. The patient had to be given several transfusions to keep her alive. (It should perhaps be noted that another patient with pernicious anemia in the same ward reacted normally on heptomin at the same time.)

The result of folic acid treatment was prompt, as is clearly seen from figure 3. The reticulocyte response was rather good. The platelets rose in the usual manner and the serum iron values decreased very markedly. The increase in the red cell count was rapid and considerable. The temperature came down to normal values and the sedimentation rate also became normal in a short time. The white cells increased but the values were rather variable and there has been a persistent tendency to leukopenia. This is especially worth noting as the leukopenia was regarded as a special indication for treatment with folic acid.

During twenty-three days the patient received 100 mg. folic acid daily perorally. After that time all treatment was stopped but the erythrocyte count continued to increase to high values (about 5.0 M.) and has remained normal for ten months

♀ Pat W. b 1890

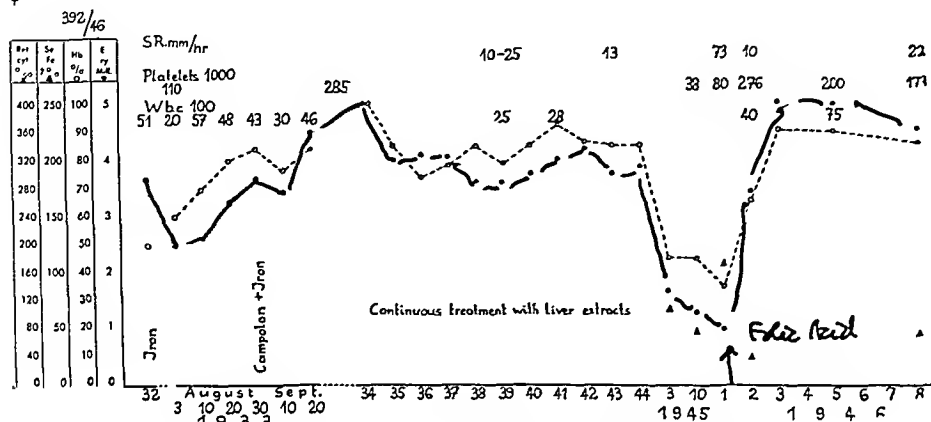


FIG. 1—Case 2. Course of blood counts with liver extract and folic acid.

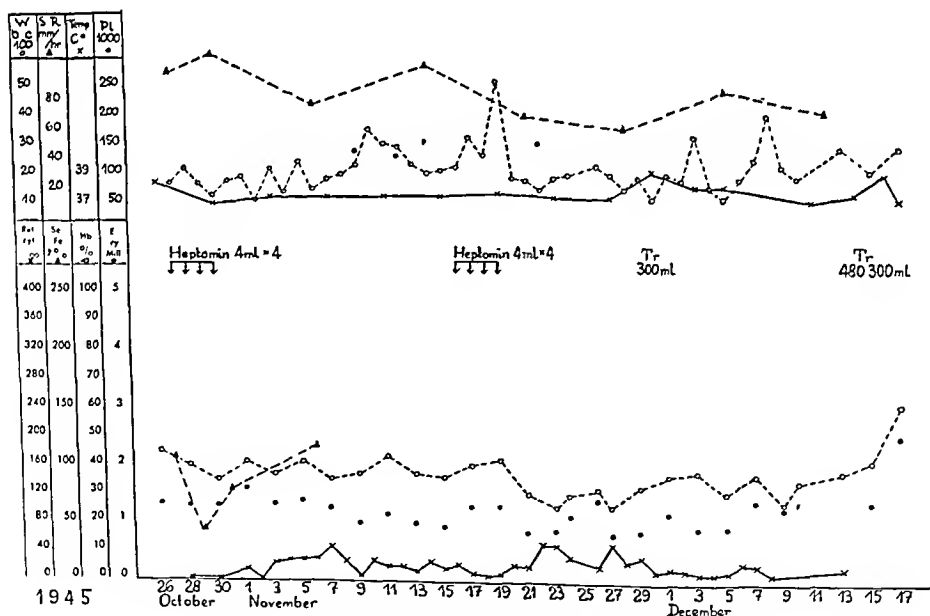


FIG. 2—Case 2. October–December 1945.

without any further treatment than iron and 5 mg. folic acid thrice daily for seven days.

*It is thus obvious that a patient who has become completely refractory against otherwise*

*potent liver extracts may react well on large oral doses of folic acid.* The long-lasting effect of this therapy speaks in favor of the assumption that folic acid itself may be stored for long times in the body or is the initiator of some secondary mechanism that keeps on promoting blood cell production in the presence of antipericious anemia factor from liver. It is of interest to note that Davis and Davidson found good therapeutic effect with proteolyzed liver after ineffective administration of liver extracts.

A chapter of special interest is the condition of the tongue in this case. Because of the fact that the lingual mucosa was refractory to all sorts of specific treatment it was assumed that the patient might suffer from some hitherto unknown defi-

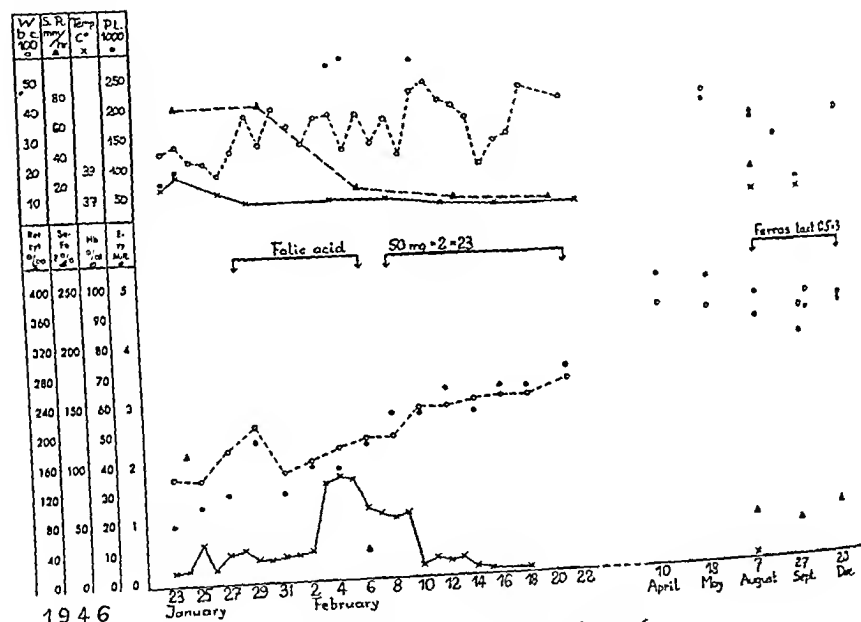


FIG. 3.—Case 1. January–December 1946.

ciency. The folic acid however was obviously not what was needed by the tongue nor was iron effective after massive folic acid administration.

Several other features should be especially noticed. At present it is impossible to tell if this patient really had a histamine-fast achlorhydria on her first examination. The amount of duodenal regurgitation was probably considerable as the samples of gastric juice were of a yellow color. When re-examined in 1945 with a special technic worked out by Hallén in order to avoid duodenal contamination, the gastric juice contained hydrochloric acid after caffeine. It is thus obvious that in this respect the patient resembled achrestic anemia. On the other hand there are no symptoms indicating an idiopathic steatorrhea and there is definitely no atrophy of the spleen.\*

\* Dr. H. Grundsell of Kristinehamn has kindly allowed me to use his data on this patient and referred her to the clinic.

Another instance of liver-refractory anemia is the following:

*Case 3.* C. B. Woman born in 1905.\* Mother died of gastric carcinoma. Otherwise there was nothing important as regards heredity. Since 1930, ulcers on both legs. About 1940, first period of diarrhea; treated with pancreatic preparations. In November 1944, ulcers on both legs with eczema. Tongue: low papillae. Hb., 63 per cent; R.B.C., 3.3 M.; sedimentation rate, 52 mm./hr. Test meal showed free hydrochloric acid. Later discontinued treatment. Re-examined in January 1946. Increasingly tired. Very pale; glossitis, dyspnea, palpitations, slightly icteric. Liver and spleen not palpable. Hb., 38 per cent; R.B.C., 1.9 M.; color index, 1.0; W.B.C., 3,300. Differential count: neutrophils 44 per cent, eosinophiles 1 per cent, lymphocytes 54 per cent, monocytes 1 per cent, reticulocytes 10 per cent. Sternal puncture: megaloblastic marrow. After histamine, free hydrochloric acid, 60 units. As the diagnosis of pernicious anemia seemed certain, the patient was given 4 + 4 ml. of a potent liver extract on 2 consecutive days. Her reticulocytosis never exceeded 5 per cent. She was later given a new injection of 4 ml. heptomin.; Hb., 37 per cent; R.B.C., 1.5 M. The result of liver treatment was regarded as unsatisfactory and the patient was sent to a hospital on January 28, where she was treated as a probable instance of aplastic anemia. On admittance her Hb. was 22 per cent; R.B.C., 1.2 M.; W.B.C., 1,700. In spite of intensive therapy with liver extracts and blood transfusions, no real improvement occurred. Severe leucopenia; at one stage not more than 420 cells. The patient was treated with blood transfusions, repeated injections of liver extracts, and nucleotide. Blood values on dismissal: hemoglobin, 40 per cent; R.B.C., 2.1 M.; W.B.C., 3,200, with improved general condition. The patient was regarded as an instance of aplastic anemia and a relapse was regarded as probable. In the end of April: hemoglobin, 50 per cent; R.B.C., 1.9 M.; W.B.C., 3,000, with some nucleated red cells. Reticulocytes, 10-15 per cent. The patient was given 2 + 1 ml. soluble liver extract (Lederle) on 2 consecutive days. No reticulocytosis. Two weeks later: Hb., 66 per cent, R.B.C., 2.7 M. On May 22: Hb., 64 per cent; R.B.C., 2.4 M. After that time R.B.C. was constantly about 2.5 M. The patient was admitted to the Medical Clinic in Upsala on June 8, 1946, as a possible instance of achrestic anemia. She felt much better than she had before. Her glossitis had disappeared and her menstruation, which had ceased during the period from December to March, had reappeared. The color of the skin was brownish, with stronger pigmentation on the face, on the hands, knees, and feet. The medial aspects of both malleoli were strongly pigmented and showed ulcerations. No pigmentation of the abdomen or in the folds of the hands. No perlèche. Tongue papillae low. Heart normal. Muscular sensibility in toes and fingers normal. Tactile and vibratory sensation normal. Serum calcium, 9.8 mg. per cent; potassium, 18.9 mg. per cent; sodium, 278 mg. per cent. Total protein, 8.8 per cent, albumin, 4.5 per cent, globulin 4.3 per cent, fibrinogen 0.35 per cent. Takata reaction positive. Formol-gel reaction, negative. Fasting blood sugar normal. Glucose tolerance test, very flat with initial value 113 mg. per cent, maximum value 140 mg. per cent, 2.5 hours after the ingestion of 55 Gr. of glucose. For blood values, see figure 4. No Jolly bodies in the smears of the peripheral blood. Sedimentation rate, 92 mm./hr. As the diagnosis of nutritional sprue seemed probable, analyses of the fat in the feces were performed in the Department of Physiological Chemistry. On June 11, 1946, the total fat was found to be 37.5 per cent, with free fatty acid, 42.7 per cent; neutral fat, 43.7 per cent; and soaps, 13.6 per cent. The analyses were repeated later on July 9. Total fat, 25.6 per cent, with free fatty acid 29.2 per cent, neutral fat 21.7 per cent, and soaps 49.1 per cent. Roentgenograms of the spinal column showed no osteoporosis. Fractional test meal: free hydrochloric acid = 30 after caffeine. The patient was given folic acid 25 mg. daily for 13 days, then 50 mg. for a week, and finally 100 mg. daily for 12 days. The total dose was thus nearly 2 gm. (The folic acid for the treatment of this patient was generously supplied by the Lederle Laboratories, New York, through Dr. Guy Clark, for which the author expresses his sincere thanks.)

The patient was discharged on July 12, 1946. The results of treatment are seen in figure 4. After a month's time without further therapy the patient returned to the hospital in very good general condition and with practically normal blood values. The blood showed: Hb., 77 per cent, R.B.C., 4.1 M.; W.B.C., 4,000. Sedimentation rate, 36 mm./hr. (fig. 4).

\* [In fact, the association of splenomegaly with a more or less refractory anemia and persistent leukopenia makes one suspect the possibility of a hypersplenic condition.—EDITOR]

## COMMENTS

Several interesting points should be noted. It is evident that the reticulocyte response was rather slight, but that the decrease in serum iron was marked. There was also a certain increase in the platelets. These three changes together indicated that the therapy may have been successful and it was expected that the red cells would improve. This was not a quick result, however, and the effect of folic acid therapy was at first regarded as a failure. The exact diagnosis in this case is not easy to make. Naturally she may be regarded as an instance of achrestic anemia according to Wilkinson. It has earlier been pointed out that there is probably a certain connection between achrestic anemia and nontropical sprue. This patient showed a few symptoms that might indicate some disturbance of this type. She had had diarrhea for several years. There was found a hyperchromic anemia with megaloblastic marrow, in spite of abundant hydrochloric acid in the gastric juice. Her serum calcium was normal and there were no signs of osteoporosis. The percentage

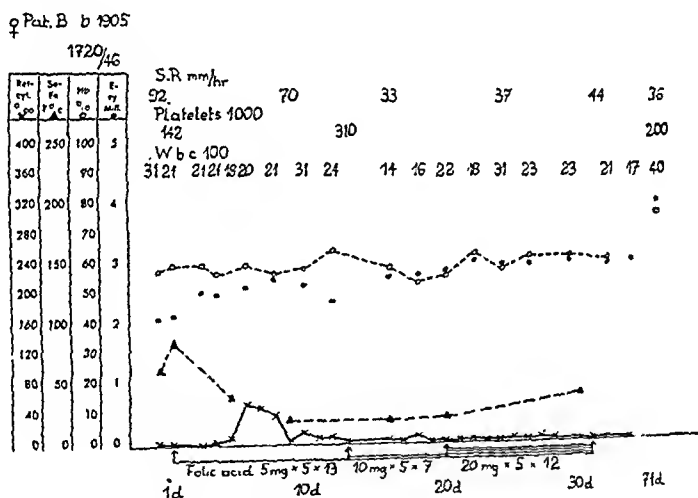


FIG. 4—Case 3. Blood counts.

of fat in the feces was rather high. This fact cannot in itself be used to prove the presence of idiopathic steatorrhea. On the other hand, the glucose tolerance test showed a very flat curve with an increase of only 26 mg. per cent after 55 Gm. glucose. Also, the curious pigmentations on the arms may be an indication of nontropical sprue. The excellent effect of large doses of folic acid perorally has lasted for several months, as in case 2.

Case 4 differed from the others in that she had a histamine-fast achlorhydria and showed no signs of leucopenia.

Case 4. Woman born in 1886.\* Hereditarily nothing of importance; previous maladies of no interest. In 1931 the patient became very tired. Various medications were without benefit. She was admitted to

\* Dr. R. Berlin referred the patient to the Clinic and kindly gave the author access to his notes on the history. Dr. Berlin recently treated a patient with macrocytic anemia, 1.6 M., and megaloblastic bone

a hospital in 1934. Clinically, typical pernicious anemia with complete achlorhydria was found and a normal remission ensued following the administration of campolon, a brand of liver extract. Injections of liver extracts were discontinued in 1937, and she was treated with heparforte perorally instead, which the patient stated she had been taking regularly. In November 1944, relapse with severe anemia (Hb., 45 per cent; R.B.C., 1.4 M.). Sternal puncture showed typical megaloblastosis. With liver extract parenterally, reticulocytosis was normal, but very slow and incomplete increase of the blood values took place (after 5 weeks Hb. was 64 per cent, R.B.C., 3.0 M.). The patient was treated with large doses of different liver extracts, 320 ml. in all during a period of 8 months with only a temporary rise of R.B.C. above 3.0 M. In May 1946, the highest red cell value was 3.4 M. No signs of complications such as carcinoma, chronic renal or liver disease or myxedema. From November 1945 to September 1946, 130 ml. of liver extracts in all were given intramuscularly.

Subjectively, the patient felt rather poorly; occasional lingual disturbance was present; no paresthesias of the legs. She felt rather nervous—"no pep." Admitted to the Medical Clinic in Upsala on October 8, 1946. On examination she appeared tired, thin, and pale. Weight, 49 Kg. Tongue reddish with atrophic

♀ Pat. C. b. 1886

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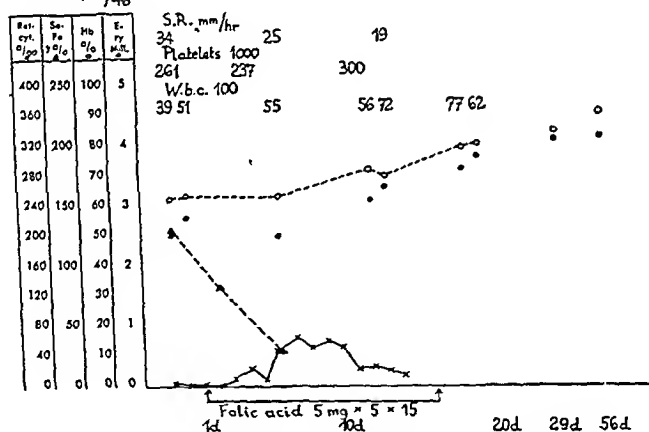


FIG. 5.—Case 4. Blood counts.

papillae. Internal organs otherwise normal except considerable decrease of vibratory sensation in the legs. No albuminuria or urobilinuria. No occult blood in the stools. For blood values, see figure 5. No Howell-Jolly bodies in the smears of the peripheral blood. Sternal puncture: scattered megaloblasts. Takara reaction normal. N.P.N., 30 mg. per cent. Histamine-fast achlorhydria. X-rays of the stomach normal.

The patient was treated with 25 mg. folic acid daily and there was rapid amelioration in the status of the tongue. The patient felt much stronger (fig. 5) and was discharged on October 30, 1946. Later examinations by Dr. Berlin showed the values: November 8, Hb. 84 per cent, R.B.C. 4.1; December 5, Hb. 90 per cent, R.B.C. 4.1 M. The patient had then taken a further course of folic acid (15 mg. daily for 3 weeks).

marrow (heptomin 8 ml. for 4 consecutive days). This had no effect. Injections of liver extracts were repeated without any results. The patient was therefore treated with a transfusion and heparforte perorally. No reaction. After a week's treatment, folic acid (20 mg. daily) was given. Maximum reticulocytosis 26.7 per cent on the fifth day. After a month, R.B.C. 4.5 M. This effect has lasted. The patient also suffers from chronic arthritis.

## COMMENTS

The diagnosis in this instance seemed to be uncomplicated pernicious anemia. In spite of this she never showed normal erythrocyte or hemoglobin values, even after massive doses of liver extracts. It was therefore regarded as desirable to try folic acid. In this instance the dosage was much smaller and lasted only for two weeks. In spite of this the response was rapid. The difference in the effect of folic acid as compared to liver extracts cannot therefore be explained solely on a quantitative basis. The result has been lasting and the patient is at present on continuous treatment with folic acid in moderate doses. The immediate effect of the therapy showed itself both as a slight increase in reticulocytes and as a very marked drop in serum iron values.

The following case belongs in the same group as case 1.

*Case 5.* Woman born in 1906.\* Nothing of importance hereditarily. As a child she was very small and delicate. She was said to have suffered from rickets. At the age of 22 she had an optic neuritis but this has never relapsed nor had she had any other symptoms of organic neurological disease. About 1943 she felt especially tired and her doctor told her that she was anemic. She noticed that her skin became darker. Examined by Dr. Grundsell in June 1945. She was quite emaciated, strongly pigmented but showed no other signs of Addison's disease. Blood pressure, 140/90. Plasma sodium, 315 mg. per cent. Sedimentation rate, 39 mm./hr.; Hb., 62 per cent; R.B.C., 3.2 M. In the beginning of May 1946, she began to have dyspeptic troubles with four to five bulky stools daily. Large numbers of fatty acid crystals were found in the feces. She was later treated for steatorrhea at the Carolina Hospital in Stockholm. In October 1946, she felt pains in her back. These pains often lasted for about an hour. Her back was quite stiff after such an attack. No menstrual disturbances of special importance.

Admitted to the Medical Clinic in Upsala on December 1946. Very thin (44 Kg.), with a wrinkled face. Skin darkly pigmented, hair and eyes also strongly pigmented. A number of brown spots were present on the arms and legs which, according to the patient, had developed after insect bites and punctures. Tongue normal. Some small aphthous ulcers in the mouth. Abdomen distended with gas, no palpable masses. Neurological examination negative. No albuminuria or urobilinuria. Prothrombin index, 76. Serum calcium, 8.8 and 9.2 mg. per cent. Total protein, 5.4 per cent. Serum iron, 71 gamma; Hb., 72 per cent; R.B.C., 3.6 M.; W.B.C., 2,800-3,200. Differential count normal. About 1.5 per cent of the red cells contained Howell-Jolly bodies. Scattered normoblasts. Reticulocytes, 6 per cent, platelets, 251,000. Sedimentation rate 42 mm./hr. Afebrile. X-rays of the spleen failed to show a definite shadow—"The spleen is probably small." Spinal column and pelvis showed no definite osteoporosis.

The feces were bulky, usually grayish in color, mostly semisolid, sometimes formed. An analysis of fecal fat was performed in the Department of Physiological Chemistry† on samples from December 7 and December 10. The total fat was 66.4, viz. 60.6 per cent; of this 38.4 viz. 27.7 per cent were free fatty acids; 36.6 viz. 30.6 per cent neutral fat and 25.0 viz. 41.8 per cent soaps. A glucose tolerance test with 44 grams of glucose showed a fasting value of 76 mg. per cent. The highest value reached was 106 mg. per cent after 45 minutes. A similar experience was made with butter (50 Gm.). Before the test the total lipids of the blood were 710 mg. per cent. There was present 125 mg. per cent/total cholesterol, 34 mg. per cent of this was free. Choline was 30 mg. per cent and lipid phosphorus 6.9 mg. per cent. Four hours after the tolerance test, the total lipids were 730 mg. per cent, the total cholesterol 142 mg. per cent/free 39/ choline 31.5 mg. per cent, lipid phosphorus 7.5 mg. per cent.

The electrocardiogram showed a rather long Q-T of 0.42 at a heart rate of 65 per minute. Because of the slightly decreased calcium values in the blood and the possible latent tetany, the E.K.G. was repeated.

\* Dr. H. Grundsell kindly referred this patient to the Clinic and gave the author access to his notes about the history.

† The analyses were kindly performed by Dr. G. Brante.

At a rate of 75 per minute, Q-T was 0.40. The patient was given 10 ml. of 20 per cent calcium (Sandoz). The heart rate was then 65/min., Q-T 0.36. Five minutes later, it was 60/min., Q-T 0.37.

The patient was treated with folic acid perorally without any obvious influence on the stools. She felt decided subjective improvement however. Her hemoglobin and red cell values after this treatment were completely unchanged. After six days of treatment the serum iron was still 90.7 per cent. Injections of folic acid (15 mg. daily) were therefore started. The serum iron decreased to 32.7 per cent, but there was no decided reticulocytosis nor any increase in red cell count. The macroscopic appearance of the feces remained unchanged and their weight was the same as before. Analyses of the fecal fat still showed 55.7 per cent total fat, consisting of 27.6 per cent free fatty acids, 45.9 per cent neutral fats and 26.5 per cent soaps.

It was thought that liver extracts might possibly have some therapeutic value after treatment with folic acid. Heptomin (4 ml.) was therefore given on 4 consecutive days. There was no reticulo- or thrombocytic response, no improvement of the red cell value and no gain in weight. The serum iron came down to very low values of 13.7 per cent. After two weeks of observation the patient was given large doses of iron because of the low serum iron value. This gave no further reticulocyte response nor did it increase the hemoglobin or the red cells. After one week, rather large doses of casein digested with enzymes from the spleen, according to a method described by Ågren, were given. This week there was a marked increase in weight of 1.6 Kg. to 47 Kg. The iron medication was stopped. As the weight decreased during this period, the patient was given no more digested casein. She was discharged with large doses of iron.

#### COMMENTS

The diagnosis is not difficult to make. The patient probably suffers from the peculiar type of idiopathic steatorrhea that is combined with atrophy of the spleen (cf. case 1). The leading symptom in this case was not so much the anemia as the steatorrhea and extreme emaciation. Atrophy of the spleen is indicated by the presence of large numbers of Howell-Jolly bodies. The connection between these findings and the other symptoms of idiopathic steatorrhea has already been discussed. The influence on the Q-T interval in the electrocardiogram by intravenous calcium therapy might indicate the presence of a latent calcium deficiency.

This patient showed a slightly hyperchromic or normochromic anemia of moderate severity. Treatment with folic acid perorally had no effect on the anemia nor did it give any reticulocytosis or decrease in serum iron. It thus seems probable that the anemia in this case was also achrestic against folic acid. Whether the suspected atrophy of the spleen plays some part in this mechanism is difficult to judge.

#### DISCUSSION

The most interesting problem in this connection is the question of why the patients 2, 3, and 4, who had lost the ability to react to treatment with liver extract, responded to folic acid. Many explanations are possible. The simplest would be that the dosage of folic acid was greater than the previous amounts of liver extract given. The difference would thus be purely quantitative. This seems improbable for two reasons. First, the dosage of liver extracts had been very considerable. Second, case 4 showed good response on doses of folic acid that are regarded as quite small. This is also in accordance with our own experience of folic acid treatment in 15 cases with uncomplicated pernicious anemia. Some qualitative difference should therefore be looked for.

Another explanation would be to assume a difference of absorption. It seems

hard to understand however that an injected substance should be less well utilized than one that is administered perorally. At least one of the cases (4), had also been treated with liver preparations per os without any effect. Therefore this explanation does not seem very convincing. The only way to look upon the matter is to regard folic acid and liver extract as different in their action. Other hypotheses could be formulated but would probably be found lacking in factual foundation. I shall therefore abstain from the further treatment of this subject until more information has been collected.

It seems obvious that case 5 suffers from a normo- or hyperchromic anemia in connection with nontropical sprue that is refractory to injected liver extract and to folic acid both in the form of tablets and injections. This would thus indicate that there may be cases of achrestic anemia both as regards the liver factor and folic acid. Whether they will respond to some other factor is not yet known but will be investigated. The question whether there are cases refractory to folic acid which respond to liver extracts cannot be answered as yet. We have been in a position to treat some 15 cases with uncomplicated pernicious anemia in relapse with folic acid. All responded well to this treatment but our experiences must be much greater before anything definite may be said. In the presence of such complications as chronic arthritis and iron deficiency, the effect of folic acid was diminished.

#### SUMMARY

Four cases of liver-refractory macrocytic anemia are described. Three were treated with folic acid perorally and reacted well.

The first was an instance of nontropical sprue with atrophy of the spleen that was diagnosed clinically and confirmed at postmortem examination. The megaloblastic anemia also became completely refractory to crude liver extracts and liver digested with gastric juice.

The second case was successfully treated with liver extracts for twelve years. After that time she suddenly became refractory with severe leucopenia. Folic acid in large doses had an excellent effect and the patient has kept relatively normal blood values for about a year without further treatment of importance. It should be noted that the morbid condition of the tongue could not be influenced by folic acid.

The third case should probably be classified as idiopathic steatorrhea. Folic acid gave a full remission of the macrocytic anemia.

The fourth case resembled typical pernicious anemia more closely, as a histamine-fast achlorhydria was present. This patient had been refractory to liver treatment but reacted well to folic acid in ordinary doses.

The fifth case was a typical instance of idiopathic steatorrhea with probable atrophy of the spleen. She had a moderate anemia that was refractory to liver extracts. Folic acid perorally and intramuscularly neither influenced the anemia nor the fecal fat.

In a series of cases of uncomplicated pernicious anemia, the immediate response to folic acid in doses of 20 to 30 mg. daily was good. Full remissions as regards

hemoglobin and erythrocytes could be expected with a total dosage of 400 to 600 mg.

Certain cases of pernicious anemia refractory to liver extract may respond to folic acid. This indicates that the two substances probably have different mechanisms of action.

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# THE ACTION OF PTEROYL GLUTAMIC ACID AND NATURAL SOURCES OF FOLIC ACID ON BLOOD DYSCRASIAS INDUCED BY SULFONAMIDE DRUGS

By H. G. PETERING, PH.D., R. A. DELOR, B.S., AND H. C. MURRAY, PH.D.

FOLIC acid or vitamin B<sub>9</sub> deficiency has been induced in the rat by feeding a purified diet containing succinyl sulfathiazole or sulfaguanidine.<sup>1,2,3,4,5,6</sup> This deficiency is corrected in either case by feeding free or conjugated pteroyl glutamic acid while the syndrome produced by sulfaguanidine is also corrected by para-aminobenzoic acid. The action of these drugs has been considered to be due to their inhibitory effect on the synthesis of folic acid in the intestinal tract of the animal.<sup>7</sup>

It has been known for some time that the more soluble sulfonamides widely used in medicine also cause blood dyscrasias in human patients which resemble those due to folic acid deficiency in rats fed sulfasuxidine or sulfaguanidine.<sup>8,9,10</sup> Some work with experimental animals has shown that sulfanilamide, sulfathiazole, and sulfadiazine cause leukopenia, granulocytopenia, and even anemia, in addition to other toxic manifestations upon prolonged administration.<sup>11,12,13</sup> Kornberg, Duff, and Sebrell<sup>12</sup> have reported that the dyscrasias in the rat due to soluble sulfonamides can be alleviated by treatment with Wilson liver fraction L and brewers' yeast.

The importance of the soluble sulfonamides in medical practice and the fact that some investigators consider that these drugs are retained in the intestinal tract in sufficient quantity to produce some bacteriostasis<sup>14,15,16</sup> which might cause the appearance of folic acid deficiency have prompted us to report our studies on the effect of crystalline folic acid, liver extract powder,<sup>17-20</sup> yeast extract, and para-aminobenzoic acid in the prevention of blood dyscrasias induced by prolonged administration of sulfanilamide, sulfathiazole, and sulfadiazine. Chronic toxicity studies reported here were undertaken, since some investigators believe they are more applicable to medical therapeutics than are acute toxicity studies.<sup>11</sup>

## EXPERIMENTAL

Twenty-eight day old weanling white rats of random sex, 45-50 Gm. in weight, were placed on the basal purified diet\*<sup>18</sup> to which in most instances was added 1 per cent of the sulfonamide drug being studied. The drug was added at the expense of the sucrose. Supplements were fed from the first day, since the object of the study was to determine the effect of certain nutritional substances on the prevention of blood dyscrasia.

Growth curves were obtained in all cases by semiweekly weighings. At the end

\* The basal diet was composed of 72 per cent sucrose, 18 per cent casein (Labco, vitamin-free), 3 per cent cottonseed oil, 2 per cent cod liver oil, 4 per cent salt (Sure), 0.1 per cent choline chloride, 0.1 per cent inositol and 200  $\gamma$  per cent of vitamin K (dissolved in the cod liver oil). Daily supplement of crystalline vitamins included: thiamine hydrochloride, 200  $\gamma$ ; riboflavin, 100  $\gamma$ ; pyridoxine hydrochloride, 100  $\gamma$ ; calcium d-pantothenate, 200  $\gamma$ ; nicotinamide, 500  $\gamma$ ; and biotin, 17.

From the Department of Nutrition, The Upjohn Co., Kalamazoo, Michigan.

of about the fifth and eighth weeks, complete peripheral blood analyses were made on all surviving rats, using conventional hematological techniques.

The antagonistic effects of the supplements on the antibacterial action of the sulfonamides was studied *in vitro* with *Str. haemolyticus* (B group Lancefield, 090 R, University of Kansas). The medium which was used was composed of edamin (protein hydrolysate) and dextrose supplemented with Speakman salts A and B, nicotinic acid, thiamine, uracil, guanine, and adenine, and adjusted to pH 6.9. Growth effects of various substances and drugs, either inhibitory or stimulatory, were measured turbidimetrically by comparison with the growth of the organism on the basal medium.

The supplements whose effects were tested in the work reported here were (1) pteroyl glutamic acid or folic acid,\* (2) liver extract powder 1:20 (L.E.P.), (3) dried yeast extract (D.Y.E.), and (4) para-aminobenzoic acid (PABA). The liver extract powder was found to contain about 50% of total folic acid per gram (42-59% range), of which about 40 per cent was free folic acid, while the dried yeast extract contained about 85% of total folic acid per gram (range 60-110%), of which about 90 per cent was in the conjugated form. Some difficulty was experienced in accurately assaying the dried yeast extract by microbiological technique due to the variability of the enzymatic digestion step.†

## RESULTS

The hematological data are summarized in tables 1 and 2, and representative growth curves of the rats are given in figure 1. An interpretation of these results is given below.

Experiment I illustrates the response obtained under our conditions on a preventive experiment with 1 per cent sulfasuxidine. It can readily be seen that 100 mg. or more of dried yeast extract daily, furnishing about 8.5% of "total" folic acid, kept the animals in almost normal condition as judged by the level of blood components, but normal growth was not attained until 200-300 mg. of D.Y.E. were fed daily. This experiment also illustrates the severe leukopenia, granulocytopenia, anemia, and growth depression produced in the rat by feeding 1 per cent sulfasuxidine in the purified basal diet, which otherwise maintains the animals in as good condition as does the stock diet. In this experiment a severe anemia was experienced by the animals receiving the drug alone, the appearance of which is variable (cf. exp. II).

Experiment II (table I) was carried out to illustrate the relative effects of sulfasuxidine and sulfathiazole in the basal diet as compared with basal and stock diets in litter mate rats. It is our experience that 2 per cent sulfasuxidine frequently is necessary to produce severe symptoms of growth depression and blood dyscrasias, which accounts for that level being used here. The data show that both drugs cause severe growth retardation, leukopenia, and granulocytopenia, although 2 per cent

\* Crystalline pteroyl glutamic acid or folic acid, supplied as Folvite 5 mg. tablets of Lederle Laboratories, suspended in water containing a trace of ammonium hydroxide.

† Suarez et al.<sup>23</sup> have reported that J. R. Totter has found a polypeptide of para-aminobenzoic acid in yeast to be a specific inhibitor of the enzyme conjugase.

sulfasuxidine appears to be somewhat more drastic in its effects on the white cell picture.

The action of 1 per cent sulfanilamide is shown in the data from experiment III, table 2. These results indicate that sulfanilamide probably causes as severe a growth depression as does sulfasuxidine, but the effect on the blood is less severe although

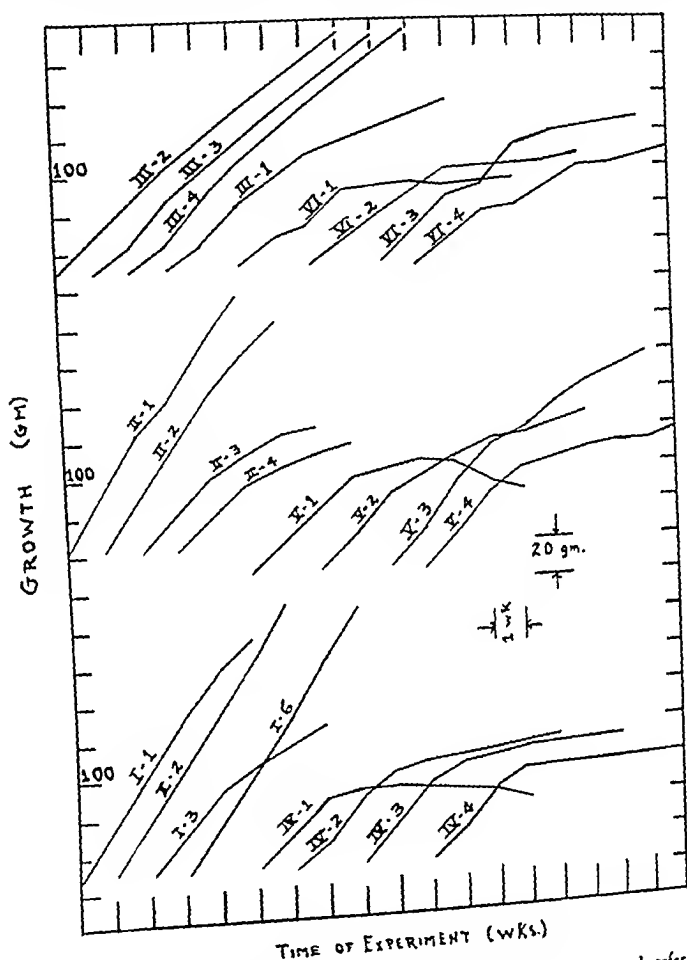


FIG. 1. Growth curves of groups listed in tables 1 and 2. The identification numerals refer to the experiment and group numbers as given in tables 1 and 2.

still quite pronounced. Synthetic folic acid (pteroyl glutamic acid), crude liver extract, and para-aminobenzoic acid all prevent the growth retardation to the same extent, though completely normal growth is not attained with the levels of supplements used.

Folic acid and liver extract in equivalent amounts completely prevent the appearance of leukopenia or granulocytopenia, although it appears that a slight ane-

is present in animals receiving these supplements. Para-aminobenzoic acid at the level used shows some beneficial effect, but permits the appearance of mild symptoms of leukopenia, granulocytopenia, and anemia.

Growth retardation by 1 per cent sulfanilamide therefore appears to be due in part only to a folic acid deficiency, since it is not so completely prevented as is that due to sulfasuxidine, even though the blood dyscrasia due to sulfanilamide can be completely eliminated by 57 or more of synthetic folic acid or an equivalent amount of liver extract powder.

The effects of 1 per cent sulfadiazine in the absence and presence of folic acid, liver extract powder, and para-aminobenzoic acid are shown in the data of experiment IV, table 2. These results show that 1 per cent sulfadiazine also produces a

TABLE 1.—Data Showing the Development of Blood Dyscrasias on Purified Diets Containing Soluble and Insoluble Sulfonamide Drugs

Group no.	Diet & daily supplement	Experimental day	W.B.C. $10^3$ / (mm.) <sup>1</sup>	Granulocytes $10^3$ / (mm.) <sup>1</sup>	R.B.C. $10^6$ / (mm.) <sup>2</sup>	Hemoglobin Gm./ 100 ml.	Hematocrit % vol.	Animals surviving Animals started
<i>Experiment I. Basal diet 1% sulfasuxidine</i>								
1	Stock	35	11.8	1.53	9.0	14.0	47	5/5
2	Basal (no drug)	35	11.5	1.50	8.6	12.8	43	5/5
3	Basal	35	5.5	0.22	4.8	11.3	39	5/5
4	Basal 100 mg. D.Y.E.	35	11.0	1.32	6.7	13.7	47	5/5
5	Basal 200 mg. D.Y.E.	35	11.8	1.53	7.7	12.5	42	5/5
6	Basal 300 mg. D.Y.E.	35	13.8	1.66	8.4	12.7	44	5/5
<i>Experiment II. Comparison of sulfasuxidine and sulfathiazole</i>								
1	Stock diet	35	10.4	1.40	7.9	15.3	41	10/10
2	Basal (no drug)	35	9.7	1.13	8.0	14.2	42	10/10
3	{ Basal (no drug) 2% sulfasuxidine	35	3.9	0.07	7.6	14.2	41	10/10
4	{ Basal (no drug) 1% sulfathiazole	35	6.5	0.24	7.5	13.8	39	10/10

evere retardation of growth and pronounced blood dyscrasias. The growth is not greatly affected by liver extract, folic acid, or para-aminobenzoic acid, but the leukopenia and granulocytopenia are eliminated by 100 mg. of liver extract powder and 57 of folic acid, the former having a more pronounced effect than the latter. 20 mg. of para-aminobenzoic acid permits the appearance of mild leukopenia and severe granulocytopenia. All supplements seem to retard the development of anemia to a significant degree.

One per cent sulfathiazole in the diet appears to be more toxic than 1 per cent sulfanilamide or 1 per cent sulfadiazine, as judged by the data shown in table 2, experiments V and VI. The anemia, leukopenia, and granulocytopenia appear to be more severe with this drug than with either sulfanilamide or sulfadiazine. Mortality is greater than was shown in experiments with either sulfanilamide or sulfadiazine.

TABLE 2.—*Hematological Data Showing the Effect of Various Supplements on the Course of Blood Dyscrasias Due to Soluble Sulfonamide Drugs*

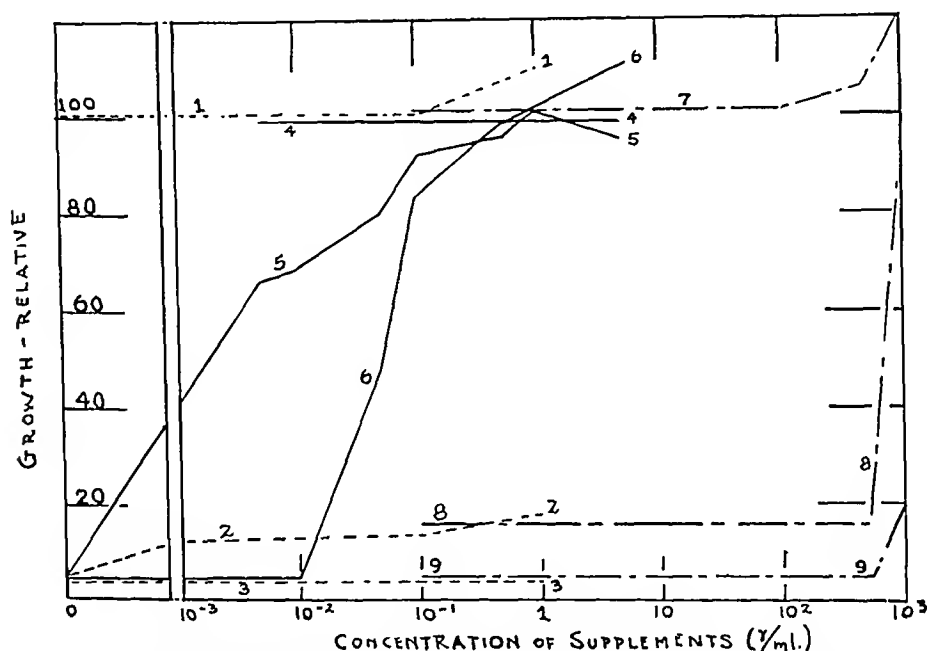
Group no.	Daily supplement	Experimental day	W.B.C. $10^3$ / (mm.) <sup>3</sup>	Granulo-cytes $10^4$ / (mm.) <sup>3</sup>	R.B.C. $10^6$ / (mm.) <sup>3</sup>	Hemo-globin Gm./ 100 ml.	Hema-tocrit % vol.	Ani-mal survival Approx. days
<i>Experiment III. Basal diet 1% sulfanilamide</i>								
1	None	35	10.0	0.80	5.9	9.3	33	
		56	7.1	0.43	6.0	8.8	29	9/10
2	5 $\gamma$ folic acid	35	16.6	2.49	6.8	10.5	37	
		56	17.4	2.44	7.4	12.2	40	10/10
3	100 mg. liver powder 1:20	35	18.4	3.31	7.1	10.8	37	
		56	18.5	2.59	7.5	12.4	39	10/10
4	2 mg. PABA	35	10.8	1.30	6.2	10.2	35	
		56	11.7	1.05	7.1	11.6	38	10/10
<i>Experiment IV. Basal diet 1% sulfadiazine</i>								
1	None	36	10.8	0.32	7.2	11.0	39	
		56	8.3	0.13	5.9	8.1	32	10/10
2	5 $\gamma$ folic acid	36	11.1	1.10	7.6	11.7	40	
		56	12.5	1.06	7.6	9.8	37	9/10
3	100 mg. liver powder 1:20	36	16.7	1.67	8.2	12.2	41	
		56	15.6	1.40	7.9	10.2	40	10/10
4	2 mg. PABA	36	11.9	0.83	7.4	11.0	39	
		56	9.9	0.50	7.2	9.4	35	9/10
<i>Experiment V. Basal diet 1% sulfathiazole</i>								
1	None	35	10.1	0.20	7.4	12.5	36	9/10
		56	3.2	0.00	3.9	6.2	22	6/10
2	10 $\gamma$ folic acid	35	16.7	1.84	8.2	13.8	39	8/10
		56	13.5	1.08	8.3	11.5	40	8/10
3	200 mg. liver powder 1:20	35	15.2	1.72	7.1	14.2	39	
		56	13.6	0.95	7.5	11.8	43	10/10
4	200 mg. D.Y.E.	35	14.6	2.15	7.0	14.1	40	9/10
		56	10.8	1.30	7.2	11.8	42	9/10
<i>Experiment VI. Basal diet 1% sulfathiazole</i>								
1	None	36	7.5	0.08	7.3	11.9	41	7/10
		56	6.4	0.06	6.5	9.4	31	7/10
2	10 $\gamma$ folic acid	36	14.3	1.86	8.5	13.3	44	7/10
		56	12.0	1.20	8.1	12.6	37	7/10
3	200 mg. liver powder 1:20	36	15.2	1.22	7.6	12.6	43	8/10
		56	16.1	1.45	7.7	13.2	38	8/10
4	2 mg. PABA	36	9.4	0.75	6.6	11.2	38	7/10
		56	10.0	0.80	7.4	11.1	35	7/10

All supplements have a small beneficial effect in preventing the serious growth retardation due to the drug, but 200 mg. of liver extract powder equivalent to about 10  $\gamma$  of folic acid seems definitely to be the best in this respect. Synthetic folic acid,

liver extract powder, and dried yeast extract effectively prevent the onset of the severe anemia shown in the negative group. Para-aminobenzoic acid has a lesser effect on the anemia.

All supplements prevent the development of the severe leukopenia and granulocytopenia found in the negative control animals, although para-aminobenzoic acid again seems much less effective than the other substances.

In an experiment not shown in the tables, the addition of high potency anti-pernicious anemia extract (10 units) or 2 mg. of para-aminobenzoic acid to folic acid has no significant effect on the course of the blood dyscrasias or growth due to



[FIG. 2. In vitro microbiological data of antagonism of various supplements to sulfadiazine, using *Str. haemolyticus*, B Lancefield (909 R, University of Kansas). Curves 1, 2, 3 are folic acid vs. 0, 10, and 100 γ sulfadiazine per ml. respectively; curves 4, 5, 6 are PABA vs. 0, 10, and 100 γ sulfadiazine per ml. respectively; curves 7, 8, 9 are L. E. P. (1:20) vs. 0, 10, and 100 γ of sulfadiazine per ml. respectively.

1 per cent sulfathiazole, but the liver extract seems to increase survival. This decrease in mortality seems also to be evidenced in other groups receiving liver extract powder (cf. table 2).

It should be noted that in the two experiments with 1 per cent sulfathiazole the relative effectiveness of synthetic folic acid, the mixture of free acid and combined folic acid in a natural source such as liver extract, and conjugated folic acid, as contained almost entirely in yeast extract, was investigated. It seems certain that both free and conjugated folic acid are effective with sulfathiazole as well as with sulfasuxidine. Any increased value of liver extract over its folic acid content must be ascribed to other materials present.

In view of the striking effect of folic acid, liver extract powder, and dried yeast extract in preventing blood dyscrasias due to soluble sulfonamides as well as in alleviating toxicity as manifested in growth and mortality and on the basis of the reports of Lampen and Jones<sup>19, 20</sup> that pteroyl glutamic acid was not antagonistic in most cases to the antibacterial action of sulfadiazine, in vitro studies of the supplements used in the animal experiments reported above were carried out, using an enterococcus which on the medium used does not require any of the supplement for good growth.

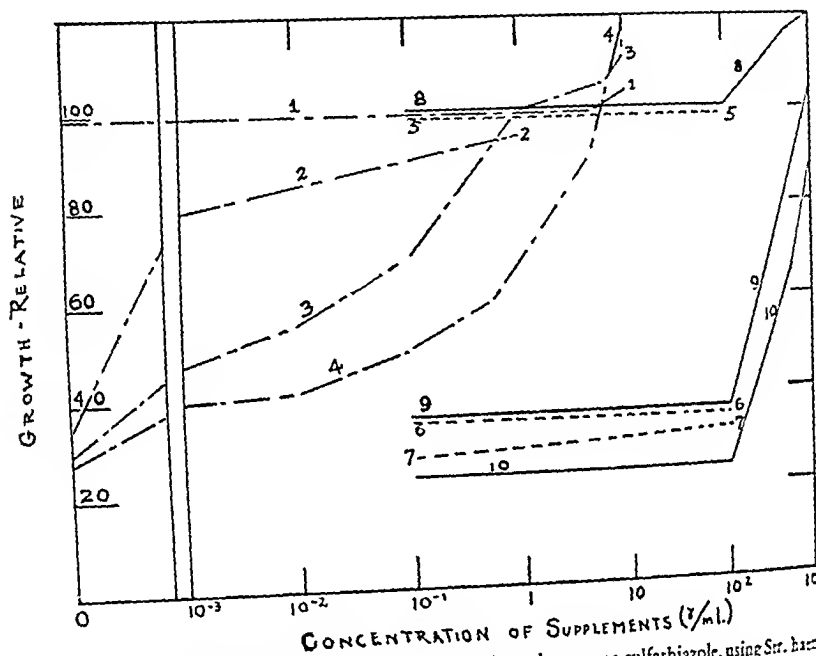


FIG. 3. In vitro microbiological data of antagonism of supplements to sulfathiazole, using *Str. haemolyticus*, B group Lancefield (090 R, University of Kansas). Curves 1, 2, 3, 4 are PABA vs. 0, 10, 100, and 1000  $\gamma$  of sulfathiazole per ml. respectively; curves 5, 6, 7 are D.Y.E. vs. 0, 10, and 100  $\gamma$  of sulfathiazole per ml.; curves 8, 9, 10 are L. E. P. (1:10) vs. 0, 10, and 100  $\gamma$  sulfathiazole per ml.

The data of these studies are shown in figures 2 and 3. They show that in large concentration liver extract and possibly folic acid have some stimulatory effect, although these are not needed for good growth on the medium used; para-aminobenzoic acid and dried yeast extract have no stimulatory effect. Furthermore, they indicate that sulfadiazine and sulfathiazole have the expected antibacterial effect, which is counteracted in stoichiometric proportion by para-aminobenzoic acid. From the same data it can be seen that relatively large amounts of liver extract, yeast extract, or folic acid have no significant antagonism to the drugs. The effect of liver extract at large concentration is considered to be due to artefact of color and turbidity caused by the liver extract itself and to a nonspecific stimulation, since the controls without drug show stimulatory growth effect at the same

concentration, and even at these high concentrations of supplements definite bacteriostasis due to the sulfonamide drugs occurs.

Thus these data confirm those of Lampen and Jones and extend it to include such natural sources of folic acid as are quite devoid of "effective" para-aminobenzoic acid.\*

### DISCUSSION

The blood dyscrasias due to the soluble (or absorbable) sulfonamides have received very little attention from the experimental point of view, at least in comparison with the attention given the insoluble sulfonamides. This is understandable in view of the value of these latter drugs in producing clear-cut nutritional deficiencies in animals not otherwise susceptible to them, and in view of a rather simple explanation of the action of these insoluble intestinal bacteriostatic agents, namely, the interference with alimentary synthesis of vitamins by bacteria. However, the action of the soluble drugs and especially their toxic action is of the highest importance, since these are the ones most widely used in medicine.

Although it has been shown<sup>15, 16, 17</sup> that significant amounts of the soluble sulfonamides remain in the gut, especially on prolonged administration, to cause some bacteriostasis, the work of Light et al.<sup>13</sup> in rats seems to show that appreciable if not adequate synthesis of folic acid occurs in rats fed 0.5 per cent sulfadiazine or sulfanilamide in a purified diet. The same authors showed that this was not true for rats fed 0.5 per cent sulfaguanidine in a similar diet. The beneficial action of folic acid, liver extract, and dried yeast extract under our conditions may be due to the fact that the folic acid not synthesized in the gut is supplied in the diet, but some unpublished data seem to indicate that the soluble sulfonamides may actually raise the animals' requirement for folic acid.

The action of para-aminobenzoic acid obviously is due to antagonism of the sulfonamide drugs. The fact that 2 mg. of PABA† daily, which was probably adequate for completely blocking sulfonamide action, was never as beneficial as the other supplements, regardless of the drug used, lends some credence to the assumption that folic acid may be required in larger than normal amounts for tissue metabolism or for normal bone marrow integrity in the presence of soluble sulfonamide drugs.

Our data tend to confirm the observations of Kornberg, Daft, and Sebrell<sup>12</sup> in that the severity of the dyscrasias due to sulfathiazole and sulfadiazine is greater and less easily prevented than that due to sulfanilamide. Mortality is also greater with sulfathiazole than it is with the other drugs in the absence of nutritional supplements.

These workers showed that liver extract and brewers' yeast had a favorable effect on the dyscrasias. Our work shows that the beneficial effects are almost

\* It is not known whether the polypeptides containing PABA have an antagonistic action to sulfonamides.

† It is estimated that 2 mg. of PABA daily maintained the ratio of PABA: sulfonamide in the animal's food at 1:50, which is much higher than the *in vitro* ratio required for inhibition of sulfonamide bacteriostasis.

entirely due to the addition of folic acid, either free or conjugated, to the diets. It is interesting to note that no significant difference between free and conjugated folic acid (as dried yeast extract and in liver powder) could be found. There is some indication in our data that liver extract powder is more beneficial prophylactically against sulfadiazine and sulfathiazole than can be accounted for on the basis of its folic acid content. This effect is most noticeable with sulfathiazole in reducing mortality, in growth response, and in preventing leukopenia.

The anemia tends to be hypochromic in the case of all the soluble sulfonamides under the conditions used, which is similar to that produced by Higgins<sup>19</sup> in rats fed promin and promizole. Higgins showed that on a diet similar to the one used here the effects of promin and promizole could be prevented and alleviated with vitamin B<sub>6</sub>.

The value of para-aminobenzoic acid in the therapy is nil, since it is contraindicated during administration of sulfonamide drugs of any kind. This fact has led clinicians to be suspicious of all B vitamins as possibly interfering with sulfonamide drug action. The work of Lampen and Jones<sup>19, 20</sup> and the data reported here on the difference between the *in vitro* action of para-aminobenzoic acid and sources of folic acid indicate that B vitamins may well be found to be valuable during sulfonamide therapy.

It seems that the quantities of folic acid even in the form of liver extract powder or dried yeast extract needed to prevent or lessen the adverse effects of soluble sulfonamides on the blood picture do not interfere with the bacteriostatic action of these drugs.\* In fact, the recent reports of Wood et al.,<sup>22, 23, 24</sup> which show the importance of blood phagocytes in supplementing the bacteriostatic action of sulfapyridine in experimental pneumonia, seem to permit the extrapolation that folic acid or liver extracts and adequate B vitamin supplementation may actually be beneficial in augmenting soluble sulfonamide bacteriostasis by keeping the circulating granulocyte concentration at a high level. (Para-aminobenzoic acid is not considered to be an essential B vitamin for animals *per se* and hence is not included in B vitamin requirement.)

The work of Wood and co-workers has shown that in experimental pneumonia sulfapyridine acts only bacteriostatically and that the bacterial invasion is overcome by normal surface phagocytosis. Furthermore, the maintenance of a high concentration of leukocytes and granulocytes during soluble sulfonamide therapy seems to be desirable as a preventive measure against the possible bacterial invasion not readily combated with the sulfonamide being administered.

#### SUMMARY

Sulfanilamide, sulfathiazole, and sulfadiazine have been fed at 1 per cent levels in highly purified diets and their effect on growth, mortality, and blood dyscrasias compared with that of sulfasuxidine.

The soluble drugs produce conditions which are similar to those produced by sulfasuxidine. The growth depression is alleviated in large measure in the case of

\* From the *in vitro* work shown in figures 2 and 3 it is estimated that the free PABA content of L. E. P. and D. Y. E. is less than 2.7/gm.

sulfanilamide and to a lesser extent for sulfathiazole and sulfadiazine by folic acid, liver extract powder, and dried yeast extract as well as by para-aminobenzoic acid,

The blood dyscrasias due to sulfanilamide, sulfathiazole, and sulfadiazine are severe leukopenia, granulocytopenia, and mild-to-severe anemia. These are uniformly prevented or the severity greatly curtailed by feeding folic acid, liver extract powder, or dried yeast extract. PABA has a lesser effect in the amounts fed.

Liver extract powder seems to have a beneficial effect on growth and mortality which is not shown by the other supplements. Both free and conjugated folic acid (as yeast extract and in liver extract powder 1:20) are active in combating the dyscrasias.

Evidence from in vitro experiments with *Str. haemolyticus* (B Lancefield) indicates that neither folic acid, liver extract powder, nor dried yeast extract in ratios to sulfonamide which are effective in preventing the blood dyscrasias will inhibit or block the bacteriostatic action of the sulfonamide drugs in vitro.

It is suggested that the action of folic acid, liver powder, and yeast extract is not wholly explained by alleviating a folic acid deficiency caused by intestinal bacteriostasis due to the drugs, but by an increased demand of the animals for folic acid in the presence of certain sulfonamides.

#### ACKNOWLEDGMENTS

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## HEMATOPOIESIS IN PANTOTHENIC ACID-DEFICIENT RATS

By L. L. ASHBURN, M.D., FLOYD S. DAFT, PH.D., AND R. R. FAULKNER, M.D.

GRANULOCYTOPENIA and anemia can be produced in experimental animals by a variety of methods. In this Institute these conditions have been produced by the administration of sulfasuxidine (succinyl sulfathiazole)<sup>1</sup> or of thiourea plus thyroxin,<sup>2</sup> and by means of deficiencies of pantothenic acid,<sup>3</sup> riboflavin,<sup>4</sup> or protein.<sup>5, 6</sup> In these studies diets of purified food materials have been employed. In experimental rats the diet of itself leads to granulocytopenia in only a very small percentage of animals.<sup>7</sup> In all of these experiments therapeutic studies indicated that a deficiency of folic acid had developed. This vitamin was shown to correct both the anemia and granulocytopenia of the animals receiving sulfasuxidine,<sup>8</sup> the granulocytopenia which developed in riboflavin-deficient animals and those given thiourea plus thyroxin, and cases of granulocytopenia which developed without anemia in pantothenic acid-deficient animals.

In view of the fact that granulocytopenia and anemia could be produced by such a variety of regimens, it seemed that the bone marrow picture in certain of them should be studied in detail in order to determine their essential identity or to characterize their differences. The status of the hematopoietic tissue and its response to treatment in folic acid-deficient and riboflavin-deficient rats have been described by Endicott, Daft, and Ott<sup>9</sup> and Endicott, Kornberg, and Ott.<sup>10</sup> The present report records similar data on granulocytopenia and anemia which developed in rats fed a purified diet deficient in pantothenic acid.

The conditions of the present experiment were essentially similar to those previously described.<sup>3</sup> The analysis of the data, particularly with respect to the quantitative study of the bone marrow, follows that used by Endicott and associates in folic acid and riboflavin deficiencies.<sup>9, 10</sup>

### EXPERIMENTAL METHODS

Albino rats of the Osborne and Mendel strain within a week after weaning were placed on a pantothenic acid-deficient diet which consisted of leached and alcohol-extracted casein, 18 per cent; crisco, 8 per cent; salt mixture no. 550 (1), 4 per cent; dextrose (Merck U. S. P.), 69.8 per cent;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.18 per cent; and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.02 per cent. Into each 100 Gm. of this diet were incorporated 1 mg. of thiamine hydrochloride, 2 mg. of riboflavin, 1 mg. pyridoxine hydrochloride, and 200 mg. of choline chloride. Each rat received a supplement twice weekly of 0.25 cc. of corn oil containing 2,000 units of vitamin A and 200 units of vitamin D (natola).

Eight rats from two or more litters were placed on the experiment at a time, usually each week. Within the week that rats were placed on the experiment,

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\* Rats given sulfasuxidine will be referred to hereafter as "folic acid" deficient.

the following determinations on the peripheral blood were made: hemoglobin (oxyhemoglobin method—Evelyn photoelectric colorimeter), hematocrit (Van Allen), erythrocyte and leukocyte counts (in duplicate—Trenner pipets), differential leukocyte count (200 cells), and reticulocyte count.

The rats were observed daily and when loss of weight, facial porphyrin stains, general weakness, or pallor occurred, leukocyte and differential counts and hematocrit determinations were made. When these procedures indicated the presence of anemia or granulocytopenia, complete blood studies as indicated above were repeated and the rat was studied in one of the following groups:\*

(a) Ten rats were killed for the study of the hematopoietic tissue in severe granulocytopenia. These rats had less than 150 polymorphonuclear leukocytes per cubic millimeter of blood and hematocrits above 30 volume per cent. The use of this hematocrit in selecting the rats for this group is arbitrary and the value somewhat low. This figure was chosen as a dividing point so that animals placed in groups (c) and (d) (hematocrit below 30 volume per cent) would have unquestioned anemia.

(b) For the purpose of evaluating the response to treatment of the hematopoietic tissue in severe granulocytopenia, a group of 22 rats (polymorphonuclears—150 or less per cu. mm., hematocrit above 30) were given daily oral doses of 100 micrograms of synthetic pteroylglutamic acid† (folic acid) and 5 milligrams of pantothenic acid. Most received in addition 10 milligrams of niacinamide daily. Ten rats treated for 4 days and 7 rats for 8 days responded by showing a rise in leukocytes in the blood to 2,000 or higher per cu. mm. Five rats did not respond to treatment in 4 or 8 days and are not considered in the analysis of the bone marrow response. Some rats died in less than 4 days and are not included in the 22 treated rats listed above. Complete blood determinations were made at the end of the treatment period. The rats were then killed for detailed study of the hematopoietic tissue. In preliminary analysis of treatment data, there was no evident difference in bone marrow response between those rats receiving folic acid and pantothenic acid and those treated with all three vitamins; hence they were combined for final analysis.

(c) A third group of 15 rats with both granulocytopenia and anemia (polymorphonuclears below 150, hematocrit below 30) were studied for comparison with group (a).

(d) A fourth group of 7 rats with uncomplicated anemia (hematocrit below 30, polymorphonuclear leukocytes above 1,500 per cu. mm.) were studied to determine the status of the hematopoietic tissue in this dyscrasia. The hematocrit value of 30, used in the selection of these rats, insured the inclusion of only those animals with well developed anemia.

The response to treatment of the hematopoietic tissues in anemia was not studied, since at the time animals were being selected for treatment the incidence of un-

\* A number of rats died during the experimental period without having developed either granulocytopenia or anemia and were discarded.

† Supplied through the courtesy of Lederle Laboratories, Inc., Pearl River, N. Y.

complicated anemia was too low to permit the accumulation of a large enough group to be of significance.

Rats were killed with illuminating gas and autopsied immediately following the final blood determinations. Marrow smears were made from the left femur by the plasma or serum dilution technic.<sup>11</sup> Five hundred to 1,000 cells were classified following Giemsa staining. The cervical lymph nodes, thymus, and spleen were cleanly dissected and weighed while wet. These tissues as well as the heart, lung, liver, kidney, pancreas, adrenals, right femur, and a portion of the thoracic spinal column were fixed in buffered neutral 4 per cent aqueous solution of formaldehyde. Bone was decalcified in 5 per cent formic acid. Tissue was embedded in paraffin and duplicate sections were stained with hemalum azure eosinate and iron hematoxylin picro fuchsin. Frozen sections of one adrenal of each rat were made and stained with oil red O.

The bone marrow was studied quantitatively by the method described by Endicott et al.<sup>10</sup> In brief, this consists of preparing 5" x 7" photomicrographs of the marrow portion of the mid-shaft, distal metaphysis, and distal epiphysis of the femur and of the body of the vertebra at magnification of 250 diameters. The area of the marrow cavity and the area occupied by active marrow were determined by analyzing the photomicrographs through a ruled transparent overlay. The proportion of active marrow to available marrow space was determined for each photomicrograph, and the average for the four areas obtained. This average was multiplied by the percentage of each type cell in the marrow smear to give an index of the total quantity of the various cells present in the marrow.

#### RESULTS

In the tables the hemograms, myelograms, and organ weights of the various groups are shown as averages and standard deviations. In keeping with one of the aims of the study, certain comparisons are made in the text of the findings in this study with those of the folic acid and riboflavin deficiencies reported by Endicott et al.<sup>9, 10</sup>

*Bone Marrow in Granulocytopenia.* The bone marrow in all granulocytopenic rats showed some degree of hypoplasia, the mean percentage of active marrow for the group being 53.1 as compared to 82.7 for controls (table 1).<sup>\*</sup> As would be expected, the decrease in granulocytes was much more marked, the mean index of total granulocytes being 5.6 as compared to 43 for controls. This decrease in granulocytes took place largely at the expense of the more mature cells, segmented forms, and metamyelocytes. These dropped from a normal mean index of 28.5 to 2.6. The erythroid cells were not involved in the hypocellularity of the marrow. In fact the index for these cells showed a rise from a normal of 25.5 to 35.0. Because of this increase in erythroid cells and the marked depletion of granulocytes, the myeloid-erythroid ratio showed an extreme reversal, from a normal of 1.93 to 0.23.

<sup>\*</sup> The data for control rats used here and in tables 1 and 2 are those given in the report of Endicott et al., reference 10.

This increased number of erythroid cells of the marrow is in contrast to the erythroid depletion found in folic acid deficiency granulocytopenia. With this exception the marrow pictures in the three deficiency states are essentially similar, both qualitatively and quantitatively.

*Effect of Treatment with Pantothenic Acid, Folic Acid, and Niacinamide on the Bone Marrow of Granulocytopenic Rats.* The detailed findings in these rats following

TABLE 1.—Hemograms and Myelograms in Experimental Pantothenic Acid Deficiency Granulocytopenia Before and After Treatment (Means and Standard Deviations)

	22 normal adult rats		10 granulocytopenic rats (pmns. below 150, hematocrit above 30)		Granulocytopenic rats treated with folic acid, pantothenic acid and niacinamide							
					10 rats treated 4 days				7 rats treated 8 days			
	Mean	S.D.	Mean	S.D.	Before treatment		After treatment		Before treatment		After treatment	
					Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Peripheral blood												
Hemoglobin (Gm./100 cc.).....			11.1	2.4	13.9	1.4	11.7	1.6	12.6	1.6	13.3	1.1
Hematocrit.....	45.6	3.9	35.5	3.4	43.1	2.5	39.9	2.6	39.2	4.3	42.9	3.3
Erythrocytes/cu. mm. in millions.....			5.302	1.603	7.887	1.911	6.717	0.837	8.006	1.082	6.394	1.177
Leukocytes/cu. mm.....	14,750	5,110	2,226	1,250	4,044	2,549	15,328	7,826	2,601	1,374	22,572	13,412
Neutrophils/cu. mm.....	3,180	1,970	64	59	108	50	6,598	5,934	86	27	13,768	13,381
Eosinophils/cu. mm.....	240	171	1 had 132, others 0		8	15	100	128	4	6	111	179
Lymphocytes/cu. mm.....	10,840	4,332	1,995	1,175	3,928	2,538	8,630	4,056	2,511	1,303	8,951	3,179
Nucleated erythrocytes/cu. mm.....	1 had 40, others 0		708	21,215	199	980	1,548	3,164	478	555	81	216
Reticulocytes, %.....			4.8	6.0	1.4	1.4	6.8	6.4	2.3	1.1	11.7	9.8
Active cellular marrow, %.....	82.7	9.12	53.1	20.7			88.6	6.1			89.1	4.4
Index of total quantity in marrow*												
Neutrophilic segmented forms & metamyelocytes (index).....	28.5	6.0	2.6	5.1			43.1	12.1			41.6	14.8
Neutrophilic myelocytes & premyelocytes (index).....	7.6	2.2	2.5	5.2			8.1	2.9			8.9	3.1
Eosinophilic granulocytes of all types (index).....	6.9	4.6	0.5	0.4			1.6	0.8			4.1	2.7
Total myeloid cells (index).....	43.0	10.5	5.6	9.8			52.8	13.5			51.6	11.4
Erythroid cells of all types (index).....	25.5	7.3	35.0	19.6			26.9	11.2			21.9	12.5
Myeloid/erythroid ratio.....	1.93	0.93	0.23	0.38			2.56	1.78			3.71	2.62
Blast cells of all types (index)....	1.57	0.51	0.3	0.3			1.1	0.4			1.3	0.7
Lymphocytes of all types (index).....	11.0	3.5	7.7	4.9			5.4	2.6			8.1	3.9
Miscellaneous cells (index)†.....	1.65	0.95	4.5	4.2			2.4	1.4			3.2	0.5

\* See text for method of determining index.

† Reticulo-endothelial cells, plasma cells, mast cells, megakaryocytes, and unidentified cells are included.

treatment are shown in table 1. The animals included in this group are those that responded by showing at the end of the treatment period (4 or 8 days) at least 2,000 polymorphonuclear leukocytes per cu. mm. of blood. At the end of the 4th day of treatment the bone marrow showed marked hyperplasia. The percentage of active marrow had risen from a pretreatment (untreated rats) level of 53.1 to 88.6. This increased cellularity of the marrow was almost entirely due to the prolifera-

tive activity of the granulocytes. The total granulocyte index rose to 52.8. The index for neutrophilic segmented forms and metamyelocytes rose to 43.1, which is considerably above the normal of 28.5. The eosinophil index did not approach normal following 4 days of treatment. The index of marrow erythroid cells after treatment was 26.9, a figure distinctly below that of the untreated animals but approximately the same as that found in the control rats. The cause of this apparent erythroid depletion is not evident, but it may be due to a crowding out of these cells. The observed drop in red cell count during treatment is in line with the decrease in marrow erythroid index. This drop in erythroid index and the marked granulocyte hyperplasia resulted in a high myeloid-erythroid ratio, namely 2.56. After 8 days of treatment the marrow showed very little further change except in cells of the erythroid series. The index for these cells dropped to 21.9, giving a myeloid-erythroid ratio of 3.71.

The marrow response in treated pantothenic acid deficiency granulocytopenia is in most respects similar to that occurring in folic acid deficiency. The only observed difference was the return of the myelocyte-premyelocyte index to normal following treatment for four days in pantothenic acid deficiency, whereas in folic acid deficiency it rose to about three times normal. From this it is evident that in the regenerative phase of the former deficiency, proliferation and maturation of granulocytes took place at a similar rate, while in the latter there is relative or actual retardation of maturation.

The marrow response in treated riboflavin deficiency is similar qualitatively to that of pantothenic deficiency but of less magnitude.

*Bone Marrow in Combined Granulocytopenia and Anemia.* The bone marrow in the rats of this group showed marked atrophy. Congestion and edema of variable degree were seen in most animals. The stroma was loose in texture and showed few or no adult fat cells. These changes were similar to those in the granulocytopenic group but were more severe. The percentage of active marrow was reduced to 31.7. The granulocytes showed extreme depletion, the mean index being 0.6. The erythroid index was reduced to 19.8 from a normal of 25.5. This drop is similar in magnitude to that occurring in folic acid deficiency, but contrasts with the erythroid hyperplasia which occurs in the granulocytopenic and anemic rats fed a diet deficient in riboflavin. The marrow granulocyte picture was essentially similar in all three deficiencies, but the granulocyte depletion was somewhat more marked in the pantothenic acid deficiency.

*Bone Marrow in Anemia.* The rats placed in this group showed a well developed anemia (mean hematocrit below 30), but no evidence of granulocytopenia (granulocytes above 1,500 per cu. mm. of blood). In spite of this, the most striking change in the marrow was the depletion of granulocytes. The index of total granulocytes dropped to 28.8. The granulocyte depletion occurred largely in the segmented forms and metamyelocyte group.

The mean erythroid index of 28.1 did not differ significantly from the normal of 25.5. There was considerable variation in erythroid indices for the individual rats. However, there was no consistent correlation between index and degree of anemia. As pointed out by Endicott<sup>9</sup> for the anemia of folic acid deficiency, here

too factors in addition to the quantity of marrow erythroid tissue must play a prominent part in the production of anemia.

*Lymph Nodes.* The cervical nodes were similar in all rats of the granulocytopenic, the anemic, and the granulocytopenic and anemic groups. Lymphoid atrophy of variable degree occurred and involved both medulla and cortex, particularly the

TABLE 2.—*Hemograms and Myelograms in Experimental Pantothenic Acid Deficiency Anemia, and Granulocytopenia and Anemia (Means and Standard Deviations)*

	22 normal adult rats		15 granulocytopenic and anemic rats, (pmns. below 150, hematocrit below 30)		7 anemic rats (pmns. above 150, hematocrit below 30)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Peripheral blood						
Hemoglobin (Gm./100 cc.).....			4.8	2.4	6.0	2.0
Hematocrit.....	45.6	3.9	17.5	7.2	20.9	6.6
Erythrocytes/cu. mm. in millions.....			2.565	1.224	3.388	1.410
Leukocytes/cu. mm.....	14,750	5,110	1,009	702	3,952	879
Neutrophils/cu. mm.....	3,180	1,970	32.0	42.3	1,656	273
Eosinophils/cu. mm.....	240	171	1	6	45	69
Lymphocytes/cu. mm.....	10,840	4,332	977	703	2,236	709
Nucleated erythrocytes/cu. mm.....	1 had 40, others 0		709	1,309	8,397	5,860
Reticulocytes, %.....			3.6	3.1	3.4	3.0
Active cellular marrow, %	82.7	9.12	31.7	20.2	77.0	13.4
Index of total quantity in marrow*						
Neutrophilic segmented forms & metamyelocytes (index).....	28.5	6.0	0.2	0.3	17.8	9.0
Neutrophilic myelocytes & premyelocytes (index).....	7.6	2.2	0.1	0.1	9.0	7.4
Eosinophilic granulocytes of all types (index).....	6.9	4.6	0.3	0.8	2.0	1.6
Total myeloid cells (index).....	43.0	10.5	0.6	1.1	28.8	14.5
Erythroid cells of all types (index).....	25.5	7.3	19.8	15.0	28.1	13.8
Myeloid-erythroid ratio.....	1.93	0.93	0.06	0.09	1.30	1.2
Blast cells of all types (index).....	1.57	0.51	0.1	0.17	1.1	0.9
Lymphocytes of all types (index).....	11.0	3.5	6.7	6.7	10.2	7.6
Miscellaneous cells† (index).....	1.65	0.95	4.5	3.7	8.6	1.0

\* See text for method of determining index.

† Reticulo-endothelial cells, plasma cells, mast cells, megakaryocytes, and unidentified cells are included.

latter. The most striking alteration in these nodes was the almost complete absence of follicles. These structures were seen in only three of the many nodes from the 32 animals, and in these the follicles were small and indistinct. Focal inflammation was seen in a few nodes. The mean weight of the nodes from the three groups is shown in table 3. Although there is considerable variation between the

node weights of different animals, the means for the three groups are not significantly different.

Following treatment for 4 days, there was marked response. Lymph node weights rose to 152 mg. as compared to 106 mg. for the untreated granulocytopenic rats. The histologic change was even more striking. The cells of the pulp were more compactly disposed, larger, and sometimes seen in mitosis. Many large active follicles were present in the nodes from more than half of the animals. After 8 days of treatment the mean weight of nodes had risen to 345 mg. However, more of these nodes showed small inflammatory foci and occasionally a large abscess was present. Histologic examination showed more evidence of lymphoid hyperplasia. Numerous large active follicles were present in the nodes of all rats. In the nodes from 1 rat treated for 4 days and from 2 rats treated for 8 days, there were few to many patchy areas of myelosis. These foci varied in size and in concentration of the granulocytes. In some the cells were mature but in most areas younger cells, including many myelocytes, predominated.

TABLE 3.—*Weight of Thymus, Spleen, and Lymph Nodes in Mg. (Means and Standard Deviations)*

	Granulocytopenic rats (pmns. below 150, hematocrit above 30)		Granulocytopenic and anemic rats (pmns. below 150, hematocrit below 30)		Anemic rats (pmns. above 150, hematocrit below 30)		Treated granulocytopenic rats			
	Mean	S.D.	Mean	S.D.	Mean	S.D.	after 4 days treatment		after 8 days treatment	
Submaxillary and posterior cervical lymph nodes.....	106	78	104	60	90	44	152	56	345	231
Thymus .....	47	15	45	25	46	27	95	25	166	101
Spleen.....	414	164	506	251	712	217	737	161	1,339	583

*Thymus.* On gross examination of rats of the granulocytopenic and the granulocytopenic and anemic groups, the thymus appeared reduced in size and consistency. The mean weights for the several treated and untreated groups are shown in table 3. On histologic examination extreme lymphoid atrophy was evident, and only very few lymphocytes could be found. The gland was made up of compactly disposed, large reticulum cells. Their cytoplasm was ample and usually deeply oxyphilic. Though comparable in weight to those of the other two groups, the thymus in the anemic rats showed less lymphoid atrophy. Although some showed atrophy of entire lobules, generally the lymphoid atrophy was patchy and incomplete.

Following 4 and 8 days of treatment, the mean weights of the glands were 95 and 166 mg., respectively, as compared to 47 mg. for the thymus of untreated rats. This increase in weight only partially reflects the lymphoid hyperplasia evident on histologic examination, for lymphocytes had increased from almost zero in untreated rats to normal concentration in most rats in 4 days and in all rats after 8 days treatment. The lymphoid cells generally were larger than normal, their nuclei were less compact, and in a number of glands mitotic activity was

evident. Whether or not the lymphoid response is directly related to the specific treatment given cannot be determined from this experiment. It is possible that the response is related to the increased food consumption which occurs during treatment.

*Spleen.* The mean weights of the spleen for the various groups of rats are shown in table 3. Histologically, in the spleen of untreated rats there was evident reduction in activity of lymphoid follicles. The cells of these structures generally were small and mature; also the normal collars of large cells were absent or thin. The pulp was very cellular in almost all spleens and comprised largely erythroid and myeloid cells of variable maturity. Hematopoiesis in this organ was considered to be moderate or marked in 26 of the 32 rats. It was of slight degree in 4 rats and minimal in 2. This degree of hematopoietic activity is greater than that seen in control rats of comparable age.

This persistence of hematopoiesis in the spleen in the presence of marked marrow depletion is in sharp contrast to the finding in the granulocytopenic and the granulocytopenic and anemic rats in folic acid and riboflavin deficiencies. In these

TABLE 4.—*Megakaryocytes in the Spleen and Bone Marrow*

Groups	Number of rats	Megakaryocytes in 100 microscopic fields		Myeloid index of marrow	Erythroid index of marrow
		Spleen	Bone marrow		
Control rats.....	8	70	165	43.0	25.5
Anemic.....	7	45	36	28.8	28.1
Granulocytopenic.....	10	15	23	5.6	35.0
Granulocytopenic and anemic....	15	3	8	0.6	19.8
Treated (4 days) granulocytopenic.....	10	74	128	52.8	26.9

deficiencies, the spleen usually showed no hematopoietic activity; when present it was of minimal degree.

Treatment for 4 and 8 days resulted in progressive enlargement of the spleen. Histologically, there was some increase in the amount of splenic hematopoietic tissue. In all animals the degree of activity was considered as marked. Lymphoid follicles showed evidence of activity. Germinal centers were commonly seen and mitoses were not infrequent.

*Megakaryocytes in the Spleen and Bone Marrow.* In spite of the somewhat increased amount of hematopoietic tissue in the spleen of rats with untreated dyscrasias, it seemed evident on histologic examination that megakaryocytes were reduced in number. To determine the validity of this impression, the number of megakaryocytes found in many microscopic fields (field diameter of 0.36 mm.; section thickness, 5-7 $\mu$ ) was totaled and recorded in terms of number per 100 fields. A similar examination of the spleens of 8 control rats and 10 granulocytopenic rats treated for 4 days was made for comparison. Megakaryocyte count also was made on the sectioned femoral bone marrow. A comparison of the findings is shown in table 4.

It is evident that as the myeloid index of the marrow decreases, there is a corresponding decrease in the megakaryocyte count of the marrow and spleen. As the marrow myeloid index reaches normal after treatment, the megakaryocytes reach or approach normal also. From this it seems clear that the factors controlling megakaryocyte production are closely related to those affecting the myeloid cells of the marrow and, conversely, that they do not appear related to the factors influencing erythrocyte production.

*Purpura.* Slightly more than 25 per cent of the rats in the granulocytopenic and the granulocytopenic and anemic groups showed intracutaneous hemorrhage. On reflecting the abdominal skin at autopsy these purpuric foci were seen irregularly scattered through the dermis. The foci were small, usually less than 1 mm. in diameter, and had sharply defined borders. Occasionally color changes in the cervical lymph nodes suggested hemorrhage, and this was confirmed histologically in 1 rat. In addition a moderate amount of blood was seen in the lymph node sinuses of 10 animals. Blood in lymph node sinuses occurred more frequently in the granulocytopenic and anemic rats than in granulocytopenic animals. In this connection it is of interest to note that megakaryocytes in the spleen and bone marrow were greatly reduced in combined anemia and granulocytopenia. In the animals with granulocytopenia alone, megakaryocytes was much less reduced. Neither purpura nor blood in lymph node sinuses was seen in the 7 rats of the anemic group. These rats showed the least reduction in megakaryocytes.

In addition to the rats noted above, purpura was seen in 2 rats that responded to treatment and in 2 that did not respond in 4 days. It was also seen occasionally in animals that died while on experiment.

*Adrenal Glands.* The great majority of adrenal glands from rats of the three untreated groups showed moderate to marked lipid depletion (28 of 32 rats). In the other 4 there was slight or no depletion. Hemorrhage and necrosis were seen in the adrenals of only 8 rats. The pattern of lipid depletion and the hemorrhage and necrosis have been fully characterized previously.<sup>12</sup> After 4 days of treatment, the lipid content of the adrenals of 7 rats was normal, slightly reduced in 2, and moderately in 1. After 8 days of treatment, the adrenal lipid content of all rats was within normal range. In both groups a few rats showed some variation from the normal distribution pattern.

*Granulocytopenic Rats That Did Not Respond to Treatment.* In this group are included 5 rats which did not show a significant rise in circulating polymorphonuclear leukocytes at the end of the 4 day treatment period. In 3 rats the thymus and lymph nodes were atrophic. Follicles were not present in the nodes, and the thymus was formed of compactly arranged large reticulum cells and was almost devoid of lymphocytes.

The bone marrow was quite atrophic. Although detailed analysis was not made, the degree of atrophy was considered to be at least as great as that seen in the untreated granulocytopenic rats.

Although the mean weight of the spleens of the "no response" rats was somewhat greater than that of the untreated group, histologically there was no evidence of stimulation of the hematopoietic or lymphoid tissue.

The lipid content of the adrenal cortex was slightly greater on the average in these "no response" rats than in the untreated granulocytopenic rats. However, since this difference is not great and the series of rats is small, it cannot be determined whether this difference is significant.

## COMMENT

When it was decided to compare in this laboratory the hematopoietic tissue of rats in which granulocytopenia and anemia were produced by three different dietary regimens—folic acid, riboflavin, and pantothenic acid deficiencies—it was assumed that these dyscrasias resulted directly from the deficiencies of the three specific vitamins. However, shortly after the experiments were initiated, treatment data from the studies of Daft et al.<sup>3</sup> and Kornberg et al.<sup>4</sup> indicated that a deficiency of folic acid was involved in all three of the granulocytopenias. In view of this relationship, from a standpoint of causation, it is not surprising that the changes in the bone marrow with respect to the granulocytes followed a similar pattern in the three different dietary regimens. The bone marrow was hypocellular and granulocytes were markedly depleted, particularly the more mature forms. In folic acid and riboflavin deficiencies, Endicott et al.<sup>9, 10</sup> did not find any evidence to suggest that in developing or fully developed granulocytopenia there occurred any stage in which there was an increase in marrow granulocytes. The same was true in the present study. This point is made in view of the fact that certain human cases of granulocytopenia are described as showing maturation arrest with hyperplasia of the stem cells.<sup>13</sup>

The status of the marrow erythropoietic tissue in granulocytopenia and in combined granulocytopenia and anemia in the three dietary regimens differs. The accompanying tabulation shows this variation.

	Marrow erythroid cells	
	in granulocytopenia	in granulocytopenia and anemia
Folic acid deficiency	depleted	approx. normal
Riboflavin deficiency	approx. normal	increased
Pantothenic acid deficiency	increased	depleted

It would not seem that this variation should occur if the anemia in all were an expression of induced folic acid deficiency. That induced folic acid deficiency is not responsible for the anemia occurring in riboflavin and pantothenic acid deficiencies is indicated by the treatment data from experiments reported by Kornberg et al.<sup>4</sup> and Daft et al.<sup>3</sup>

Lymphoid hypoplasia or atrophy of the spleen, lymph nodes, and thymus seen in these deficiencies cannot be definitely related to the specific vitamins in view of the fact that this atrophy occurs in inanition, a condition seen in all three of the deficiencies. Further work will be necessary to answer this question.

Extramedullary myelosis involving lymph nodes is uncommon. In the present study it was observed in 3 of 17 treated rats. This suggests both a great physiological need and an intense stimulating action of the vitamin therapy for granulocyte production.

As pointed out by Daft et al.,<sup>3</sup> there are similarities between the marrow atrophy seen in this study and the panmyelophthisis of rats described by György and Goldblatt.<sup>14</sup> It appears that they found a somewhat greater degree of general marrow atrophy than was observed in the present study. In fact the term "panmyelophthisis" could not be applied to our group of granulocytopenic rats. In this group, the marrow granulocytes are markedly reduced but erythroid cells were present in greater than normal numbers. A reduced platelet count was a feature of the panmyelophthisis of György and Goldblatt. In the present study platelet counts were not made. However, the greatly reduced number of marrow and splenic megakaryocytes suggests the probability of a reduced number of circulating platelets.

#### SUMMARY

Rats fed a purified diet low in pantothenic acid developed granulocytopenia and anemia singly or in combination. In the former, the marrow showed marked depletion of granulocytes, particularly of the more mature cells, and a slight increase in erythroid cells. In combined granulocytopenia and anemia the granulocytes of the marrow were still further reduced and the erythroid cells were also depleted. Marked reduction in the number of megakaryocytes occurred both in the granulocytopenic and in the granulocytopenic and anemic rats. Purpura was noted grossly in about 25 per cent of the rats of both groups. In anemia without accompanying granulocytopenia the marrow granulocytes showed slight to moderate depletion, whereas the erythroid index (mean) was not significantly lowered. Megakaryocytes were moderately reduced.

Lymphoid tissue—spleen, thymus, and cervical nodes—showed atrophy of variable degree, most marked in the thymus.

Adrenal glands showed marked depletion of cortical lipoids and rarely hemorrhage and necrosis.

Following treatment with combined folic acid, pantothenic acid, and niacinamide, granulocytopenic rats responded by showing a prompt rise in lymphocyte and polymorphonuclear leukocyte count, marked granulocyte response of the bone marrow, increased splenic hematopoiesis, lymphoid hyperplasia, and increased lipid content of the adrenal glands.

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# THE USE OF PHOTO-ELECTRIC TURBIDOMETRY IN THE DETERMINATION OF RED CELL COUNTS, HEMATOCRITS, AND HEMOGLOBIN

By J. H. WHITLOCK, PH.D.

RECENT medical literature contains several reports of the use of photo-electric turbidometers in the determination of blood counts and hematocrits. Although many accounts praise the accuracy and rapidity of the method, most of them have described paradoxical phenomena which have made technicians slow to adopt the technic as routine.

However, the paper by Richardson (1943) in which he summarizes the literature and makes his own contribution in the physics of turbidometry helps resolve many of these paradoxes. In essence the accepted views are to the effect that when a beam of light is passed through a turbid solution some of the light is absorbed and some is scattered by the particles in suspension. The net effect is that the extinction of light is proportional to the total projected area of the particles in suspension if there are not so many of them as to cause overlapping of their shadows.

Since agitation of properly prepared suspensions of blood in saline produces no fluctuation of the galvanometer of a photo-electric device, it seems obvious that the total projected area is a constant for any one suspension. If we could divide the total shadow by the number of cells interfering with the light, we could find an average shadow. Such a statistic would reflect both the size and shape of the red cell in suspension. It should be related to the form factor which is so difficult to determine in conductivity measurements of cell volume (Ponder, 1944).

Determination of the average shadow for various normal and abnormal bloods seemed to be an excellent way of predicting the reactions of the turbidometer to such suspensions. Accurate scale models of blood cells were made from measurements given by Ponder (1934) for sheep, rabbit, and human blood and by Haden (1940) for various abnormal human bloods. These were placed in a special holding device and projected by sunlight on to paper of uniform thickness. Each model was then oriented through  $90^\circ$  of obstruction of the light between maximum and minimum obstruction and a tracing made of the shadow at each  $10^\circ$  interval. Due precaution was taken to keep the drawing surface perpendicular to the light source and parallel to the axis of rotation of the model. Each shadow tracing was then weighed and multiplied by the cosine of the angle of the model with the light. The sums of such products for each model were divided by the sum of the cosine values. The value thus resulting was in turn divided by the weight-area value for the paper to give the average shadow for the model. The use of the cosine weighting device was necessitated by the fact that all the angles of obstruction of the light were not in fact equally probable. It is rather remarkable that the figures obtained after such extended calculation agree closely with those obtained by assuming that the average shadow was an ellipse whose major axis was the diameter of the cell and whose minor axis was the average of the thickness and the diameter.

From the Department of Veterinary Parasitology, N. Y. State Veterinary College, Cornell University, Ithaca, N. Y.

Figure 1 illustrates the relationship between the average shadow and cell volume from the data given by Ponder (1934) and Haden (1940).

Note that with the data given by Ponder the shadow volume relationship seems to fall along a straight line indicating that for some species at least it is a constant. Note also that for the abnormal bloods a characteristic deviation from the normal constant is observed. The disparity between the normals given by Ponder and Haden is due to the fact that the latter author based his measurements on dried smears while the former used saline or plasma suspensions. This figure explains why Shohl (1940) found that with microcytic anemia the turbidometer overestimated the cell volume while with pernicious anemia the turbidometer underesti-

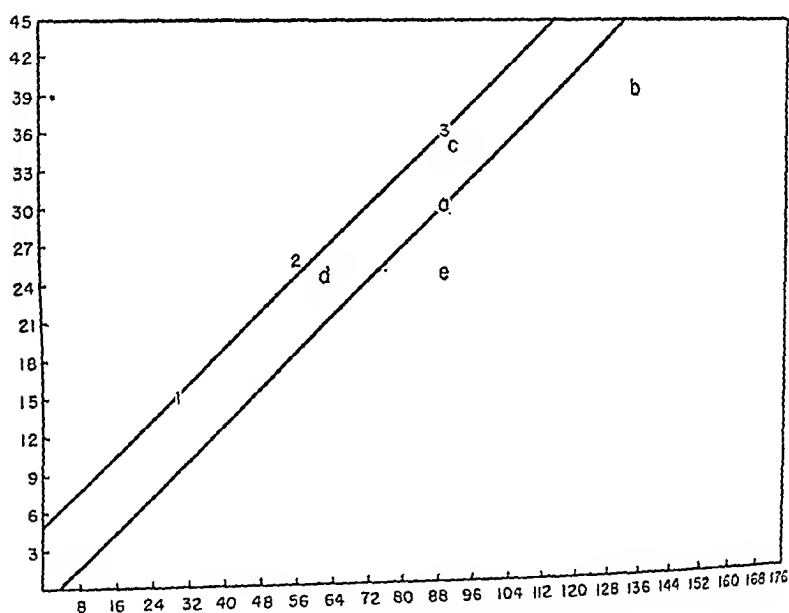


FIG. 1. AVERAGE SHADOW AND CELL VOLUME

- |                     |                                  |                                |
|---------------------|----------------------------------|--------------------------------|
| 1 = Sheep (Ponder)  | a = Normal human (Haden)         | d = Microcytic anemia (Haden)  |
| 2 = Rabbit (Ponder) | b = Pernicious anemia (Haden)    | e = Hemolytic jaundice (Haden) |
| 3 = Human (Ponder)  | c = Obstructive jaundice (Haden) |                                |
- Abcissa: cell volume      Ordinate: average shadow

mated it. It also explains why Blum (1945) found that the cells in hypotonic saline showed a reduced shadow, whereas with hypertonic saline the shadow was increased. Changes in the maximum diameter of the cell have a disproportionately large effect on the shadow and do not of necessity parallel the volume changes.

#### METHOD

For our work we have used the Klett-Summerson photo-electric colorimeter, an automatic pipetting device, Sahli pipets and a modified Ringer's solution. The

automatic pipetting device discharged 5 cc. of the Ringer's solution\* into the Klett-Summerson tubes. Twenty cu. mm. of heparinized blood were added and the whole shaken in an eccentric shaking device for 10 or 15 minutes. For calibration of the turbidometer readings, counts of the cells in suspension were made using the same hemocytometer chamber throughout. Since it was statistically desirable to count as many squares as possible, eighteen squares from each blood sample were photographed with a Leica camera and photomicrographic attachment. These were later projected with a Novex projector and counted. Nine squares were photographed in each chamber. In some of the later experiments duplicate samples with 5 and 10 cc. of diluent were made. In these only nine fields from one chamber were recorded for each dilution. For calibration against hematocrits, horse and sheep samples were run in Wintrobe tubes for two hours at from 3500 to 4000 rpm. Packing seemed to be complete at this time and speed. Cow samples did not pack even after six and one-half hours.

In preliminary experiments, duplicate samples of hemolyzed and nonhemolyzed blood were run with the green, the red, and blue filters supplied with the machine. As was to be expected, readings† were highest with the blue filter, but the hemolyzed blood was as high or higher than the nonhemolyzed. With the green filter (the proper one for spectrophotometric determination of hemoglobin) the turbidity reading was higher than the hemolyzed sample. The difference between the two was of the same order of magnitude as the red filter turbidity reading. Hemolyzed blood (oxyhemoglobin) did not deflect the galvanometer at all with the red filter. It seemed probable therefore that the red filter turbidity reading was due chiefly to the scattering effect, and the extinction of light due to the absorption by hemoglobin was essentially nullified when it was used.

## RESULTS

Figure 2 illustrates the results obtained from 46 parallel determinations of red cell counts and red filter turbidity readings using anemic and normal sheep blood. The standard errors of the counts ran from 2 to 4 cells. It is obvious that this is inadequate to account for all the deviation from the regression. In part of the data figured, the turbidity readings and counts were made with 5 and 10 cc. of diluent. There was a marked tendency for counts from the same individual to fall in the same zone with respect to the regression line. This would indicate that a considerable proportion of the spread is due to variations in cell volume between different individuals. In other words, the accuracy of the technic using the turbidometer alone is limited by the variation in cell volume and by the accuracy of the pipetting technic. Figure 3 illustrates 56 parallel determinations of hematocrit and red filter turbidity with sheep blood. No practical way presented itself of determining the hematocrit error. Deviations from the line are due to three factors: pipetting error,

\* Formula of Ringer's solution: NaCl 0.9%, KCl 0.042%, CaCl 0.024%, NaHCO<sub>3</sub> 0.01%. Gower's solution, recommended by other workers, causes marked hemolysis of sheep erythrocytes.

† Since the dial of the Klett-Summerson machine is calibrated in logarithmic units, all references to readings in this paper refer to such units. With most photo-electric devices, the dial readings will have to be transformed to logarithms in order to confirm the reported data.

## PHOTO-ELECTRIC TURBIDOMETRY

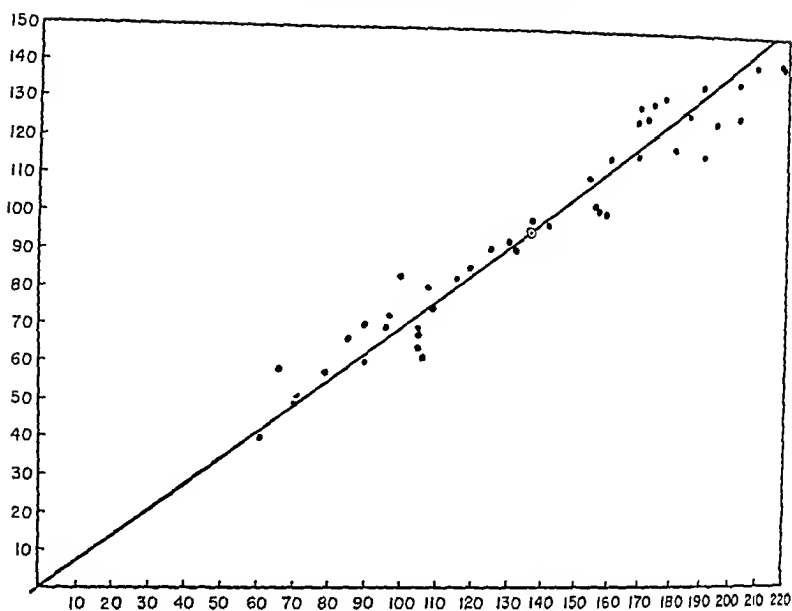


FIG. 2. RELATION BETWEEN TURBIDOMETRIC READING AND COUNTS

Abcissa:  $N/62,500$ -count

Ordinate: turbidometer reading

○ = Mean

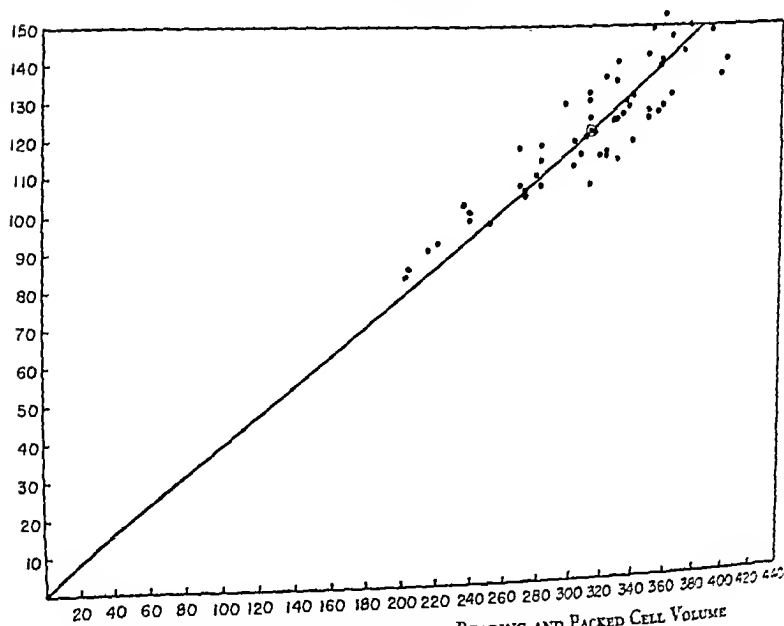


FIG. 3. RELATION BETWEEN TURBIDOMETRIC READING AND PACKED CELL VOLUME

Abcissa: vol. in cc. per thousand

Ordinate: turbidometer reading

○ = Mean

hematocrit error, and shifts in the volume shadow relationship. If we equate the regressions in figures 2 and 3, the average cell volume for these sheep can be calculated. It equals 28.1 cu.  $\mu$ .

In 44 determinations of the relationship between the hematocrits and red filter turbidity readings of anemic and normal horse blood, a distribution similar to figure 3 was obtained. The regression coefficient\* for the horse blood was 2.557 and for the sheep blood was 2.554, which offers some substantiation for the belief that with some mammals the cell shadow/volume relationship is a constant. With 10 samples of cow blood the regression coefficient was 2.903. Whether this was due to our inability to get the cells to pack properly or to some fundamental difference in the shape of the bovine blood cells could not be determined.

The possibility that turbidometry with a green filter could measure hemoglobin was first explored by Drabkin and Singer (1939), who concluded that with cell suspensions the total extinction of light was not simply equivalent to the logarithm of the extinction of light due to absorption by hemoglobin plus the logarithm of the extinction of light due to the scattering effect. However, this conclusion does not seem of too great practical importance. The important problem to be solved is: are the values obtained by the difference between the green filter and red filter turbidometry on a single sample intimately related to the hemoglobin concentration of that sample?

Drabkin and Singer (*op. cit.*) indicate that the absorption of light by hemoglobin is nullified at 630  $\mu$ . If we consider that their readings at this point represent the extinction due to the scattering effect of the red cells and subtract these values from values obtained on the same suspensions at 540  $\mu$ , the differences are related linearly and closely to the hemoglobin values given by the same authors.

In our present work we have accumulated 56 determinations of red and green filter turbidity readings and parallel acid hematin determinations on sheep and horse blood. In these data the acid hematin readings were equal to the green filter turbidometric readings minus the red filter turbidometric readings with a standard deviation of 9.2 points. That the agreement was so close to the acid hematin readings is probably due to some peculiarity of the machine. With other types of machine the relationship is probably with some other function of the oxyhemoglobin (which seems to be the pigment measured by turbidometry). However, this fact is by itself not proof that the difference is really a measure of hemoglobin concentration. Accordingly the individual deviations from the regression of the red filter on the green filter readings and of the regression of the red filter on the acid hematin readings were determined. These were then coded by addition to eliminate the minus signs. If the difference between red and green filter reading measured the hemoglobin, the deviation should correspond with the deviation on the same sample of the red filter readings on the acid hematin. A regression equation was then calculated to determine if the deviations were independent. Submitting the results to analysis of variance we find:

\* Theoretical regression coefficients =  $\frac{\text{mean hematocrit}}{\text{mean galvinometer reading}}$

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total.....			
Regression.....	55	11687	
Deviation from regression.....	5	6235	6235*
	54	5452	101

\*  $F = 62.2^{**}$  (Snedecor, 1940).

Obviously, then, the difference between the red and green filter readings is intimately correlated with the hemoglobin concentration, or, in other words, there is here some evidence that the green filter turbidity reading may represent a composite of the scattering effect of the red cell plus absorption due to hemoglobin.

The variances of the different regression equations which indicate the accuracy of the technic are as follows:

Regression	Variance
Red filter on count .....	112
Red filter on hematocrit.....	701
Green filter-red filter difference on acid hematin.....	84.5

It is not illogical to suppose that the much larger variance in the hematocrit data is inherent in the hematocrit. The best estimate of the error in the technic itself is provided by the other two variances. The sources of error in the cell count data have already been discussed. The errors in the hemoglobin determination by turbidometry are of such a nature that extensive exploration of their source is possible. The potential sources of error in such determinations are: (1) the error associated with changing the filter, (2) a potential systematic error associated with the pipetting machine, since it had to be calibrated for each change of solution, (3) the machine error, (4) the pipetting error.

In obtaining the data for the relationship between the green filter turbidity and hemoglobin, the filter was changed once each day between each series of red filter readings and green filter readings. It was necessary to allow the machine to recondition itself to the change of filter and to reset the zero mark. If there was marked error in this change, analysis of variance between and within the day should demonstrate it.

ANALYSIS OF VARIANCE OF EFFECT OF CHANGING FILTERS ON REGRESSION OF RED FILTER ON GREEN FILTER READINGS

Source of variation	Degrees of freedom	Sum of squares	Mean square
Deviations from regression .....	54	635	
Between days.....	6	108.4	18.06
Within days.....	48	526.6	10.9

The F test for these data is nonsignificant, indicating a small error in changing the filter.

The pipetting machine was calibrated once each day for the saline and once each day for the N/10 HCl. Analysis of variance of the regression of acid hematin readings on the green filter minus the red filter readings yielded the following information.

<i>Source of variation</i>	<i>Degrees of freedom</i>	<i>Sum of squares</i>	<i>Mean square</i>
Deviations from regression . . . . .	54	4565	84.54
Between days . . . . .	6	542.5	90.33
Within days . . . . .	48	4022.5	83.5

Apparently the technic of calibrating the pipetting machine was adequate.

To estimate the other sources of error a special experiment was devised. Four Sahli pipets were labeled, four containers filled with one discharge of saline from the pipetting machine, four with two, four with three, and four with four. These dilutions were repeated with N/10 HCl. The pipets were then assigned at random to each complete set of eight containers. Twenty cu. mm. subsamples of blood from the same individual were then placed in each container and mixed thoroughly. Red and green filter turbidity readings were recorded for each saline sample and acid hematin for the acid solutions.

From the data resulting, the standard deviation of the regression of the red filter on green filter readings was 1.64, demonstrating a negligible machine error. Analysis of variance of the between and within pipet error was as follows:

<i>Source of variation</i>	<i>Degrees of freedom</i>	<i>Sum of squares</i>	<i>Mean square</i>
Total . . . . .	28	399	14.2
Between pipets . . . . .	3	157	52.3*
Within pipets . . . . .	25	242	9.68

\*  $F = 5.40$ .

This demonstrates that the pipet error is large and important and that accurately calibrated pipets will be a great asset to the technic. Since many grossly inaccurate pipets were eliminated from our stock by a simplified recalibration technic before these experiments were ever started, the lack of accurate glassware is convincingly demonstrated.

## DISCUSSION

Analysis of the present data and the literature allows fairly accurate delineation of the areas of usefulness of blood turbidometry. As long as the cells in suspension do not deviate markedly from the normal in shape or in volume, figures obtained by blood turbidometry can be translated directly into counts or hematocrits with considerable accuracy. In interpreting counts of normal cells, the only important errors in such translation are the pipetting error, which need not be larger than that in the standard technic, and the error due to normal variations in cell volume. Fatigue errors, errors due to inaccuracies of the counting chamber, and errors due to the Poisson distribution are thereby largely eliminated.

The standard hematocrit technics are not so accurate as the counting technics, yet translation of figures obtained by turbidometry into hematocrits can be made with comparable accuracy.

If the red blood cells are abnormal in shape and/or volume, translation of turbidometric figures into counts and hematocrits becomes an inaccurate procedure. However, it should be noted that deviations from the normal are accurately reflected in the turbidometric reading. Such error as is present is inherent in the process of translation and not in the reading itself.

Therefore, the chief use of the turbidometer is as a screening device to separate normal from anemic individuals. This it should do with considerable accuracy and with a marked saving of the technician's time.

Ponder (1935), using a green filter, has reported results which would indicate that turbidometry, as used in his experiments, is grossly more inaccurate than routine counting technics with normal bloods. This is not confirmed in the present data. Part of the discrepancy in conclusion is probably due to the fact that he used a green filter which introduces the variable of hemoglobin concentration into the data. As far as inspection of our data can demonstrate, the hemoglobin concentration and cell volume vary independently within certain limits in normal animals. Likewise Ponder (*op. cit.*) indicates that he expects a  $\pm 3$  per cent error (expressed as standard error) in his counting technic. Berkson (1944) demonstrates that the true error (expressed as twice the standard deviation) of the typical routine red cell count is more likely to fall within limits of  $\pm 16$  per cent.\* Even with perfect technic and glassware the irreducible field error (due to the dispersion of cells in the field) is of the order of 8.2 per cent (twice the coefficient of variation) for a count of 500 cells. (The usual number counted to arrive at a determination of five million cells per cubic mm.)

Turbidometry alone will not diagnose the type of anemia from which a patient is suffering. Where turbidometry indicates a deficiency of blood elements, two supplementary independent measures of cell volume and shape are needed for exact diagnosis. Since they are standardized, most workers will use the red cell count and hematocrit as the supplemental technics. However, there is reason to believe that other technics may be developed which are more rapid and perhaps more accurate. One such technic consists of examining the cell suspension in ether-washed and alcohol-washed chambers with a Haden-Hausser erythrocytometer. This gives a measure of the cells in suspension as they are and as spherocytes and provides an estimate of anisocytosis. However, such a technic is not yet practical.

As illustrated in figure 1, the deviation of the actual from the expected red count and hematocrit is in itself an aid in the diagnosis of anemia. Thus, in the leptocytic anemias, turbidometry will overestimate the count and hematocrit, whereas in the spherocytic anemias they will be underestimated. Since the deviation of the turbidometric readings is characteristic, once the diagnosis of anemia is established, the response to treatment can be followed by turbidometry alone.

Determination of hemoglobin by the difference between green and red filter turbidity readings rests upon a shakier theoretical basis than does the use of the red filter turbidometry alone. Definitive confirmation of its accuracy can only come from work upon a large series of hypo- and hyperchromic anemias. Unfortunately, these are not available to us clinically at present. However, thus far we have justification for assuming that green filter turbidometry represents a composite of scattering plus hemoglobin absorption. Except in the case of high normal readings, however, there is probably little justification for translating the green filter galvanometer reading into counts and hematocrits as Blum (1945) suggested. In perni-

\* [This figure is for routine red cell counts as done by various technicians in hospital laboratories with uncalibrated pipets, chambers, etc.—Editor.]

cious anemia, the enlarged hemoglobin component tends to cancel out the deficient cellular component due to spheromorphism when the green filter is used alone. On the other hand the disparity between the relatively high green filter reading compared with the relatively low red filter reading with this type of anemia should be very suggestive of this disease. It is not inconceivable that turbidometry alone could diagnose mild cases of this disease. Similarly, in hypochromic microcytic anemias, the cellular component is increased because of the leptocytic condition, whereas the relative hemoglobin content is reduced. Again the potential disparity between the two may be of considerable diagnostic aid. Even if determination of hemoglobin by turbidometry does not prove sufficiently accurate, the area of the relationship between red filter turbidometry and hemoglobin concentration may yield considerable data of diagnostic importance.

Each photo-electric device used in blood turbidometry must be calibrated in order to translate the readings back to numbers with which clinicians are familiar. Such calibration for counts should be done with both 5 and 10 cc. of diluent and on as many individuals as possible. The result of such calibration can be listed in tabular form to facilitate translation. Hematocrit calibration is easy in horses and sheep but is difficult in cattle, due to the failure of the erythrocytes of the latter to pack adequately. Calibration of a photo-electric device for determination of hemoglobin by turbidometry should probably be done directly, i.e., the difference between the red and green filter readings determined for a blood sample whose hemoglobin content has been previously determined by some accurate chemical method. Since the pipets at present available are not highly accurate, they should be recalibrated, and in addition the green filter-red filter difference should be determined on a number of samples of the standard blood.

The fact that both theoretical considerations and our present experiments indicate that the shadow-volume relationship is a constant in many species is of considerable interest. One possible explanation for the present data which supports such a view is that the cells become crenated or spherocytic as a result of the action of the saline. However, many of the suspensions were examined for evidence of crenation without ever finding any great amount. In addition the fact that ether-washed and alcohol-washed chambers filled with the same suspension usually gave a consistently different reading in the Haden-Hausser erythrocytometer is further evidence that the cells in suspension were relatively unchanged. The assumption that the suspending media distorts the cells relatively little allows us to explain with a simple unifying theory the results obtained in the present work as well as the peculiar phenomena observed by Blum (*op. cit.*) and Shohl (*op. cit.*). The author, of course, cannot defend the proposition that the cell in saline is the same as the cell in the circulating blood, but it seems reasonable to assume that if the saline produces any changes they are of such a nature that they are consistent with the measurements of Ponder (*op. cit.*) and Haden (*op. cit.*). The absence of agitation effect in suspensions of blood in saline in the photometer is not evidence per se of crenation or of induced spheromorphism. Since so many cells in the suspension are in the path of the beam of light, lack of fluctuation of the galvanometer merely indicates that the total shadow is unaffected by the speed at which

the cells are gyrating or rotating. As a matter of fact, a good indication of the average shadow from the models used could have been obtained by setting them to rotating rapidly in front of light sensitive paper and determining the periphery of the true average shadow by the midpoint of maximum and minimum darkness of the developed paper. It must also be noted that some accuracy has been sacrificed in the Klett-Summerson instrument so that minor fluctuations might not be apparent.

Ponder (1935) offers some proof of this linear relationship between shadow and volume by demonstrating a linear relationship between average shadow and opacity constants for the blood of man, sheep, rabbit, ox, and cat. In this case, the opacity constants represent figures necessary to make turbidometric reading—cell count curves coincide for the different species. Since, in such translation, the volume is an assumed constant, the relationship is thereby demonstrated.

### SUMMARY AND CONCLUSIONS

1. Blood turbidometry is recommended as a screening technic for distinguishing between anemic and nonanemic individuals.
2. Blood turbidometry must be supplemented by other technics for an exact diagnosis of the type of anemia. However, with such help it makes its own contribution to the accuracy of the diagnosis.
3. Blood turbidometry alone would seem to be capable of following an anemic individual's response to therapy once the proper diagnosis is established.
4. Determination of hemoglobin concentration by turbidometry appears as a distinct possibility. However, further investigation is necessary to validate its utility.
5. Evidence is accumulated that the shadow-volume relationship is a constant in several species.

### ACKNOWLEDGMENTS

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# A CORRELATION STUDY ON WHITE BLOOD CELLS

By JEROME MARTIN PINES, M.A.

A CORRELATION study was made at the Woodrow Wilson General Hospital in Staunton, Virginia, between the number and volume of white blood cells

TABLE 1.—Correlation of 11,485 cases between white blood cells per cu. mm. and white blood pack (mm. per 100 mm. tube) at Woodrow Wilson General Hospital, Staunton, Va., from October 1, 1943 to September 30, 1944

White Blood Pack (mm.)  
(100 mm. Tube)

White Blood Cells per cu. mm.

Midpoint of Class	1,000	2,000	3,000	4,000	5,000	6,000	7,000	8,000	9,000	10,000	11,000	12,000	13,000	14,000	15,000	16,000	17,000	18,000	19,000	20,000	21,000	22,000	23,000	24,000	25,000	over 25,000
11,485	3	20	29	258	875	1,609	1,969	1,946	1,493	1,158	708	503	307	183	129	72	68	32	30	19	19	13	11	3	5	
over																										
2.5	8																									8
2.5	3																			1						2
2.4	0																									
2.3	0																									
2.2	2																				1					1
2.1	5																1			1		2				1
2.0	36											1			1	2	2	1	3	5	4	2	3	2	3	7
1.9	7												1			1		2		1	1		1			
1.8	31											2	2		1	7	4	4	4	3	1	1	2			
1.7	16									2		1	1		2		1	1		1	2	3	1			1
1.6	15										1		2		2	1	2	2	1	2	1	1				
1.5	128			2		1	1	3	6	10	12	11	8	10	9	22	9	5	1	5	1				2	1
1.4	30						1	2	1	2	2	2	4	5	4	3		2		1	1					
1.3	79							1	1	3	3	6	5	8	11	10	11	8	2	4	1	2	2	1		
1.2	337				1	2	3	2	15	17	30	39	57	50	50	30	14	12	6	7	1					1
1.1	531						6	9	21	26	76	114	108	83	35	24	15	5	1	3		1		2	1	1
1.0	2024					9	26	67	142	283	380	442	274	200	100	53	30	4	6	4	1	2			1	
0.9	1259					3	18	85	137	258	298	250	124	47	25	10	2	2								
0.8	1792				1	12	82	210	391	455	344	176	70	28	13	7	1	1	1							
0.7	1859				1	22	134	344	488	464	252	87	40	19	5	1	1	1								
0.6	1562		1	3	44	172	405	480	276	102	54	15	10													
0.5	1576		2	8	103	390	474	303	167	64	29	13	11	4	4	1	1									
0.4	130				6	44	48	12	11	6	2	1														
0.3	33		1	4	7	13	3	1	1			1														
0.2	19		1	11	3	1		1	2																	
0.1	3		1	2																						

in 11,485 cases, covering the period from October 1, 1943, to September 30, 1944. A routine blood test was performed on each patient admitted into the hospital.

From Quartermaster Purchasing Office of New York, New York City.

Grateful acknowledgment is made to Dr. David R. Morgan and Dr. Erwin D. Zeman for making the data available.

The results are shown in table 1 and figure 1. The coefficient of correlation between number and volume of white blood cells is 0.75. Since different samples lead to varying results, the validity of the coefficient increases with the number of cases included in a study. With the large number of cases included in this study, the chance is 142 to 1, or nearly certainty, that the coefficient of correlation will fall between  $0.75 \pm 0.112$  or 0.7388 to 0.7612, all of which are high coefficients.

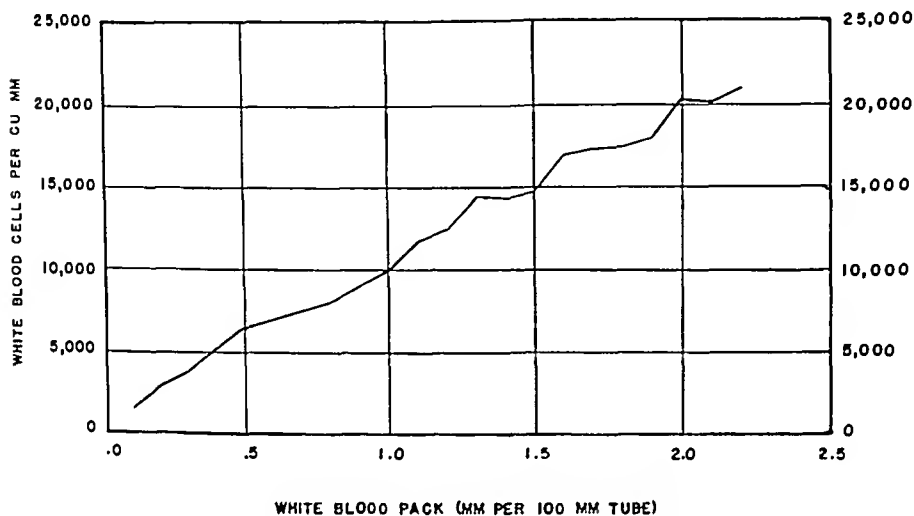


FIG. 1. Relationship between white blood cells per cu. mm. and white blood pack (mm. per 100 mm. tube).

The ranges of number and volume of white blood cells may be observed from the table and also the impracticability of ascribing any one value of number to a corresponding value for volume. For each, there is a range of the opposite. The high coefficient of correlation, however, definitely establishes a high degree of relationship.

# METHOD

## NEW METHOD OF PRECISE LIQUID MEASURING. NEW BLOOD DILUTING DEVICE

By N. BEN-TOVIM, M.D.

**L**IQUID MEASURING and diluting operations for medical and other purposes by means of the usual gage tubes, such as pipets and burets, may be performed in a very precise and convenient manner by using a simple device described herewith.

*Principle of the Method.* The general idea is to arrange a movable obturator beneath the bottom end of a gauge tube, consisting essentially of a piece of rubber fixed to a rigid support, and to provide the obturator with such mechanical means for operating it as to allow the degree of the obturation to be finely varied and exactly adjusted.

*Schematic Realization (Fig. 1).* A pipet (1) is fixed to a rigid support (3) by clasp (4). An obturator (14), made of a square rigid frame over which a small rubber tube has been slipped, tightly closes the opening of the tube by slight pressure, which is produced by a traction spring (20). It is mounted on the lower end of a vertical rod (15) on the top of which rests a lever (22) which facilitates the production of small downward and upward movements of the obturator. (It is evident that other modalities of mechanism, e.g., a micrometric screw system, can be imagined for operating the obturator.) At its upper end the pipet has a rubber tube (5) which may be compressed by means of a leaf spring (12) operated by a screw (13). The tube (5) is connected to a rubber bulb (8) by means of a rigid tube (6). This has an opening (9) closed by a shutter (10), which is pressed against the tube by a spring (11).

*Manner of Using the Device. A. Measuring a liquid.*

### Method 1

1. Draw in the liquid until somewhat above the mark.
2. Close pipet with obturator and compress tube (5) by screw (13).
3. Press gently on the lever until liquid has lowered *exactly* to the mark.

### Method 2

1. Put a drop of liquid on the middle of the obturator.
2. Replace the obturator in such a manner that the end of the pipet dips in the drop and then decompress tube (5).

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3. Press gently on the lever until the liquid has risen *exactly* to the mark.
- B. Diluting a liquid.
1. After an exact quantity of the liquid has been obtained within the tube (closed by the obturator) wash off any excess of it left outside upon the obturator.
  2. Uncover opening (9), compress bulb (8), release shutter (11) and bulb (8) (the bulb remains compressed).
  3. Dip the lower end of the tube (which is always closed by the obturator) in the diluting liquid and press gently on the lever until the mixed liquid has risen exactly to the upper mark.

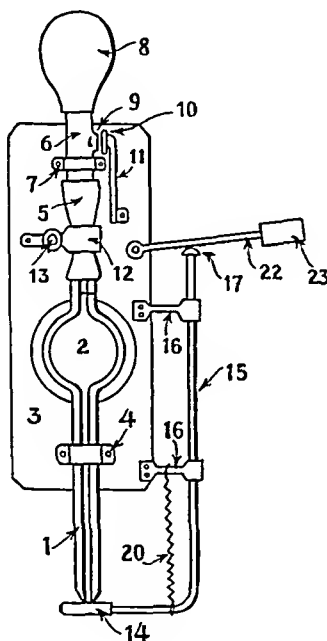


FIG. 1

#### FUNCTIONS AND ADVANTAGES OF THE OBTURATOR

1. *Regulator.* By lowering the obturator slightly we create a very narrow slit between it and the bottom end of the tube through which a given liquid can enter or leave the tube. The speed of the liquid current is proportionate to the dimensions of the slit and oscillates in exactly the same manner as the position of the obturator with regard to the bottom of the tube. This is a very delicate and precise mechanism. The current is instantly stopped at the moment of the complete obturation of the tube.

2. *Separator.* There is no residual drop at the bottom end of the tube. The excess of liquid remaining upon the obturator is separated by it from the liquid inside the tube and can simply be washed off. With "mixing pipets" into which two liquids are sucked in one after the other in order to be mixed together, no loss of liquid

occurs from the pipet into the second liquid before one begins the aspirations because of this separating function of the obturator. Likewise it is possible to discharge one liquid into another by minute quantities by dipping the end of the obturated buret into the liquid where the titration is performed (microanalysis).

3. *Secludor*. Two or more pipets can be combined into one unit with common means for producing variations of pressure in them, each pipet being quasi-insulated or secluded from the whole by its individual obturator.

4. *Receptor*. When the quantity of liquid to be measured is only a small drop, one can advantageously put this drop upon the obturator and have it sucked in therefrom.

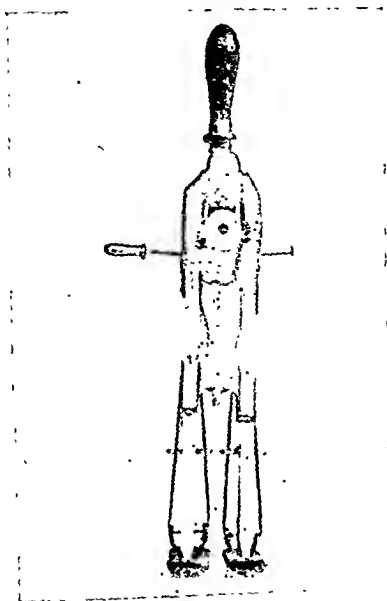


FIG. 2

5. *Obturator*. Prevents loss of liquid from the tube (when transported or shaken). Insures stability of the liquid column in the tube, thanks to the obturation and the absence of rubber tubes for aspiration; there are no traces of liquid upon the walls of the tube above the mark when using method 2. It is possible to interrupt the aspiration and continue it afterwards in order to chase air bubbles. The upward movement of the liquid is easily stopped exactly at the upper mark in mixing pipets, because in the described system gravity is counterbalanced by the unremitting aspiration, and aspiration and inertia are instantly bridled by the obturation.

The blood diluting device (*hemacytometer*). It comprises both usual pipets for leucocytes and erythrocytes count arranged upon one common support and having common means for raising and lowering the pressure in them and each has an individual obturator and lever (fig. 2). For laboratory purposes it is possible to include in the instrument a great number of pipets.

## RECAPITULATION OF ITS PRINCIPAL ADVANTAGES

1. A very simple mechanical system making use of common pipets.
2. A combination of both pipets in one unit, allowing the performance of both diluting operations partly in a parallel manner and partly simultaneously.
3. Mechanical aspiration and discharging of the liquids.
4. Close manual control under the continuous observation of the operator.
5. Highly precise adjustment of the liquid level.
6. Washing off instead of wiping off the excess of liquid at the ends of the pipets.
7. Automatic obturation of the pipets at the end of the adjustment, preventing loss of liquid and allowing shaking and transporting under good conditions.
8. The possibility of using the device as a "receptor," especially when taking blood drops from little children.
9. Easy and rapid operation, excluding the habitual failures and accidents and shortening substantially the blood diluting procedure.
10. Hygiene.

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# CASE REPORT

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## CONGENITAL HEMOLYTIC ICTERUS IN A NEGRO FAMILY

By E. G. GOODMAN, M.D., AND B. R. CATES, M.D.

IN A recent article by Stragnell and Smith<sup>1</sup> a Negro family was studied in which three members showed definite evidence of chronic hemolysis and the presence of spherocytes in the blood. Sickling preparations were negative. This stimulated us to check the hospital records and review the literature on the subject. According to the standard textbooks of hematology, congenital hemolytic jaundice in the Negro is extremely rare. Wintrobe<sup>2</sup> mentions 1 case in a Negress of "undoubtedly mixed blood." In 1945 Scherer and Cecil<sup>3</sup> described a 14 year old Negro girl with hemolytic anemia. The family was investigated. A maternal uncle and maternal grandmother, both tan in color, showed microspherocytes in blood smears. Her father and paternal uncle showed no anemia and had normal fragility tests, but a slightly increased icteric index and a low grade reticulocytosis were present.

The congenital hemolytic anemias usually show a racial predilection. In general, the Mediterranean races are subject to anemia with target and oval cells, the Negro race to sickle cell anemia, and the white race (exclusive of the Mediterranean group) to hemolytic anemia with spherocytosis. Whenever any of these diseases appears in a racial group other than that in which it is usually described, the question of racial admixture must always be considered.

Recently we had the opportunity of studying a Negro family with pure Negroid features that showed all of the characteristics necessary for a diagnosis of congenital hemolytic icterus with spherocytosis as usually seen in the white race.

We believe it is worth while to report such cases, especially since splenectomy is of definite value in this disease whereas in Mediterranean target-oval cell anemia and sickle cell anemia removal of the spleen does not appreciably alter the course of the disease.

### CASE REPORT

G. T., a 22 year old colored married female, was seen in the Duke Hospital Dispensary on August 2, 1946, complaining of cramp-like upper abdominal pain of one week's duration. The initial episode of abdominal pain occurred seven months before her visit, when she had sharp right upper quadrant pain which lasted for about an hour and was relieved by nausea and vomiting. At that time her local physician discovered that she was anemic and that the spleen was enlarged. She was admitted to another hospital where two transfusions of blood together with antianemic treatment were given. The upper abdominal pain was quite typical of gallbladder colic and occurred at irregular intervals up to the time of the severe episode which brought her to this hospital.

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The family history was interesting in that the patient's father died of "anemia," one sister died of "malaria" at the age of 15 years, and another sister had been anemic most of her life and had required several transfusions. One sister gave a history of gallbladder colic and another was asymptomatic but when studied here was found to have anemia, jaundice, and an enlarged spleen.

The general physical examination revealed nutrition and development to be adequate. Complexion was black (see fig. 1). Superficial lymph nodes were not palpable. The head was symmetrical, without exostosis or other bone deformities. There was definite icterus of the sclerae. There was a cardiac systolic murmur heard best over the pulmonic area. The blood pressure was 120/68. On abdominal examination, the spleen was both visible and easily palpable, the tip being felt just to the left of the umbilicus. The liver border could not be palpated. The extremities showed no ulcerations, cyanosis, or edema.



FIG. 1

Examination of the blood showed a hemoglobin of 8.4 Gm., or 54 per cent. R.B.C. 3,250,000 per cu. mm. W.B.C. 7,000 per cu. mm. Differential formula revealed mature polymorphonuclears 67 per cent, band forms 5 per cent, eosinophils 1 per cent, basophils 2 per cent, small lymphocytes 8 per cent, large lymphocytes 9 per cent, and monocytes 8 per cent. The reticulocytes numbered 4.7 per cent. The M.C.V. was 75 cu. micra, M.C.H. 25 micromicrograms. The fresh blood showed a marked degree of hemagglutination at room temperature. There was no evidence of sickling. There were many small, dense red cells, quite typical of spherocytes. A fragility test showed the patient's cells to begin hemolyzing at 0.66 per cent saline and to be complete at 0.36 with a control showing hemolysis to begin at 0.46 and to be complete at 0.34. Bone marrow was aspirated from the sternum by puncture. There was a marked erythroblastic hyperplasia with 117 nucleated red cells to 100 leukocytes.

*Splenic Puncture:* A small amount of material was aspirated from the spleen through a No. 16 needle. This showed a mixture of small, large, and early lymphocytes. No abnormal cells were found.

The blood Kahn, Kline, Wassermann, and Mazzini tests were all strongly positive. The Van den Berg reaction was indirect with a total bilirubin of 3.2 mg. per cent. The total serum protein was 8.1 Gm., albumin 4.2 Gm. per cent, globulin 3.9 Gm. per cent, giving an A/g ratio of 1.1. Urine examination was negative, the urobilinogen value (Wallace-Diamond) being 1:20. Oral cholecystogram showed the gall-bladder to concentrate the dye well. There were small areas indicative of nonopaque stones.

TABLE I.—Blood Values Before and After Splenectomy

Date	Hgb.	R.B.C.	W.B.C.	Ret. %	Serum Bilirubin	Fragility	Spherocytes
	g.				Mg. %		
8-20-46	8.4	3.25	7,000	4.7	3.3	.66-.36	Present
8-23-46	7.8	3.06	9,000	9.1		.62-.42	Present
8-27-46	8.8	3.36	7,700	11.2			
8-28-46	Given 4 500 cc. transfusions preoperatively and postoperatively.						Splenectomy, cholecystectomy
8-30-46	15.0	5.77	16,600	2.4		.64-.28	Present
9-3-46	14.1	5.31	10,900	2.9	.83	.64-.28	Present
9-6-46	14.2	4.59	5,800	1.5	.6		
10-1-46	12.3	4.70	8,000	1.5		.64-.28	Present

TABLE 2.—Investigation of Family

Name	Relationship	Hgb.	R.B.C.	W.B.C.	Ret.	Serum Bilirubin	Urine Urobilinogen	Fragility	Spherocytes	Spleen	Gallstones
		g.			%	Mg. %					
G. T.	Patient	8.4	3.25	7,000	4.7	3.3	Increased	.66-.36	Present	Enlarged	Present
Age 22											
L. G.	Sister	13.0	4.37	14,500	10.0	3.38	Increased	.56-.38	Present	Enlarged	None
Age 32											
E. B.	Sister	11.1	4.31	10,400	11.7	1.94	Increased	.70-.40	Present	Enlarged	Present
Age 36											
M. A.	Mat. niece	14.4	4.80	6,450	1.0	<5%	Normal	.46-.26	None	Neg.	None
Age 22											
I. G.	Brother	17.2	5.28	6,650	2.7	<5%	Normal	.46-.32	None	Neg.	None
Age 28											
J. B.	Brother	15.8	5.23	5,350	1.5	<5%	Normal	.48-.32	None	Neg.	None
	Sister	Unable to contact—"anemic most of life." Has had several transfusions.									
	Father	Died of "anemia."									
	Mother	Living and supposedly well.									
	Sister	Died of "malaria" at the age of 15 years.									

*Course in the Hospital:* Spinal fluid examination showed no evidence of central nervous system syphilis. Quantitative serologic studies on the peripheral blood showed a titer high enough to rule out a false positive reaction. The patient was, therefore, given an intensive course of penicillin treatment. On August 28, 1946, a splenectomy and a cholecystectomy were done. An accessory spleen, measuring about 4 cm. in diameter, was discovered in the transverse mesocolon and removed. Before, during, and after operation the patient was given a total of 2,000 cc. of citrated blood.

*Pathologic Report:* Gross examination.—The spleen weighed 610 Gm. and measured 21 x 12 x 4 cm. The capsule was somewhat thickened and was covered by a fresh organizing fibrinous exudate. The cut surface of the spleen presented a congested appearance. The Malpighian bodies were particularly prominent. There was an increased amount of fibrous tissue in the trabeculations. On microscopic examination

the splenic pulp was markedly engorged by the erythrocytes, but conversely the sinusoids were empty and stood out very prominently. The reticulo-endothelial cells showed a great deal of hemosiderin pigment. The Malpighian hodies were widely separated and appeared to be reduced in number because of the great engorgement of the splenic pulp. The findings were thought to be characteristic of those seen in congenital hemolytic icterus. The gallbladder was distended by dark green bile and contained approximately ten small pigmented calculi.

The patient's postoperative course was uneventful and she was discharged from the hospital September 7, 1946. On a return visit she was doing quite well and her blood values were relatively normal (table 1).

*Investigation of Family:* Five members of the family were brought to the hospital for study. The results are shown in table 2. Two sisters were found to have anemia, reticulocytosis, spherocytosis, jaundice, increased fragility of erythrocytes, and enlarged spleens. One had gallstones. Two brothers and a maternal niece showed no evidence of disease. One sister who could not be located was said to be anemic and had been transfused on several occasions. A sister died of "malaria" at the age of 15 years and this could have been a hemolytic crisis. The father died of "anemia." No more definite information could be obtained. The mother is living and supposedly well.

### DISCUSSION

Three members of a Negro family, all females, were studied and showed all the criteria necessary to make the diagnosis of congenital hemolytic icterus with spherocytosis. After the diagnosis was established in the first case, splenectomy was performed, following which all evidence of increased hemolysis disappeared, although the spherocytes remained in the blood and the erythrocyte fragility remained about the same as before splenectomy. The gallbladder was also removed as it contained stones and gallbladder colic was the primary complaint on admission. The marked hemagglutination, which was present at room temperature when the patient was first seen, disappeared after several days; but the cells still showed auto-agglutination when the blood was cooled to 4°C.

Congenital hemolytic icterus with spherocytosis occurs in the Negro race, although it is rare. The hospital records were examined carefully and no previous cases in the Negro were found. From the reported cases, the disease seems to be more common in the female. The disease itself may be quite benign and give no symptoms unless complications occur. Cholelithiasis is one of the common complications which cause the patient to seek medical advice.

Geographically, the family reported here was from South Carolina, the one reported by Stragnell and Smith from "Carolina," and the family studied by Scherer and Cecil from Virginia. The sexes of the reported cases in the Negro are as follows: Wintrobe—female; Scherer and Cecil—female; Stragnell and Smith—1 male and 2 females (brother and sisters); Goodman and Cates—3 females, giving a total of 8 cases with 7 females to 1 male.

### SUMMARY

1. A Negro family is reported in which 3 sisters were found to have congenital hemolytic icterus.
2. All members of the family observed showed strong Negroid characteristics. There was nothing to suggest an admixture of white blood.
3. The literature was reviewed. Including the 3 reported in this paper, 8 cases have been reported. The disease may be more common in the female as the reported cases show 7 females to 1 male.

4. Congenital hemolytic icterus, on rare occasions, occurs in the Negro race and recognizing it as such is helpful since splenectomy is of distinct value.

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## EDITORIAL

### THE "ANEMIA" OF NEURASTHENIA

#### "Tallqvist" Anemia

MANY PEOPLE believe that hematology is concerned solely with purely material things which can be looked at and measured. It seems strange, therefore, that one of its most important problems is that of the neurasthenic woman under treatment for "anemia." Recognition of the problem and its intelligent handling is often more important than making the diagnosis of an obscure case of leukemia.

Be he general practitioner or highly restricted specialist, a significant bulk of the doctor's practice is made up of the neurasthenic woman. She complains of lack of energy and easy fatigue. If she entertains a few friends at dinner she is exhausted for a week. She is particularly "low" in the morning and a night's sleep leaves her anything but refreshed. She is beset by headaches, vague dizziness, ill-defined pains, palpitations, and menstrual disorders, so much so that she is often incapable of carrying on the simple activities of the household. Visits to successive doctors' offices result in such diagnoses as sinus disease, hypothyroidism, ovarian dysfunction, arthritis, low blood pressure, and anemia. Treatment includes nasal sprays, thyroid extract, estrogens, pelvic operations, and various and sundry antianemia preparations, including liver extract, iron, vitamin B complex, folic acid, and numerous combinations of these.

Inquiry reveals that the "anemia" has been under vigorous treatment, particularly with liver extract, for from six months to fifteen years. The diagnosis of anemia has usually been made by the Tallqvist hemoglobin scale, which almost always seems to give readings of 60-65 per cent. Often without further ado, the patient is placed on a series of injections of liver extract which are given daily, three times weekly, or weekly. Characteristically, each injection is followed by a quick upsurge in vitality, which wears off in a few days. Surprising responses take place in the hemoglobin and red cell values, but characteristically the patient states that she can tell when the hemoglobin level drops off a few points by a well defined reduction in vitality which develops. The improvement which follows a series of injections is usually short lived, and sooner or later the patient develops reactions to the liver extract or may even become skeptical of the necessity for continued injections.

Fatigue dominates the clinical picture and is out of all proportion to the general appearance of good health. The patient is almost always of the charming, highly feminine type with soft skin and smooth, rounded arms. Beneath the rouge, there is a sallow complexion, but the palms and mucous membranes have an excellent color. The tongue is completely normal. There is no loss in vibratory sensation. A few ecchymoses are often present on the thighs and arms. The blood pressure reading is usually between 90 and 100 systolic and a careless reading of the blood pressure often gives values of less than 90—and thus a diagnosis of low blood pressure.

As determined by a well calibrated photoelectric colorimeter, the hemoglobin values give readings of 10.9 to 15.0 Gm. (75-90 per cent). The red cell counts correspond, giving a color index of one, with a mean corpuscular volume of between 85-100 cubic microns. The leukocyte count is often at the low side of normality, between 5,000-6,000 per cu. mm. and the granulocyte level varies between 60-70 per cent. Should the basal metabolic rate be determined, it is often found to be between -15 and -25 per cent.

That the normal hemoglobin value for women may be at levels of 10.9 Gm. or even less, corresponding to red cell counts of 3.5 to 3.8 M, is not generally known. This is particularly true, it would seem, in the neurasthenic type, in which the constitutional make-up of sluggishness, lack of drive, and low energy is associated with a low metabolic function of the entire body, i.e., low blood pressure, low basal metabolic rate, etc. The disability that these women (and some men) complain of can hardly be a function of their somewhat lowered hemoglobin concentration and is indeed out of all proportion to that of a true case of anemia, in which there may be unusual vigor even at a level of 40 per cent hemoglobin.

The various physical and laboratory studies give no hint as to the cause of the weakness, which is almost certainly "nervous" and constitutional in type. This is usually hereditary, but may be the result of continued environmental difficulties. Although nonorganic, one would hardly classify the symptoms as imaginary, since there is a real lack of energy and at times complete disability. Most patients have come to rely on their frequent injection treatments for "anemia" as veritable props. To take away the diagnosis of an organic disease such as pernicious anemia, and substitute for it that of a nervous disorder or a constitutional nervous weakness may leave the patient completely disappointed or even shocked. The patient's physician may also be skeptical. Suppose the patient really has pernicious anemia, wouldn't it be dangerous to discontinue liver extract? And besides, what harm can there be in continuing the injections?

The harm is in the development of a prop, in the treatment of one symptom, or piece of laboratory data (oftentimes in error) rather than in the treatment of the whole patient. The intelligent physician wishes neither to delude himself nor his patient and is therefore always on the alert for the true state of affairs. The diagnosis of anemia must first be ruled out, and here, after the blood counts, a gastric analysis is perhaps first in order. Almost always, free HCl is found either with or without the use of histamine. Should HCl be present, the patient can be told categorically that there is no need whatever for further liver extract injections. In the occasional case, HCl is completely lacking, and here one must choose between one's clinical judgment as to the character of the case and an isolated laboratory test. Once the positive diagnosis of neurasthenia has been made, it has been my practice to discontinue all antianemic preparations and to observe the patient at intervals of approximately three months. At these times, careful inspection of the tongue, tests of the vibratory sensation and of the knee jerks are performed, and blood counts are carried out.

Almost always, the blood remains either at the same levels as on the first occasion or even shows improvement!

Some sort of treatment is, of course, required. The first and most important, and one which the patients appreciate, is frankness. An extended discussion is made of the nature of neurasthenia, its hereditary and constitutional character, its lack of relation to organic disease and its tendency to remit and relapse. The importance to the patient of removing as many "props" as possible and of walking on one's own hind legs is stressed. Adjuvant therapy which is often helpful is the use of small doses of dexedrine or benzedrine, which, when administered in the morning, give the patient a "lift" and a new sense of energy. Small doses of phenobarbital at night may be helpful if the patient is unusually fearful.

The problem of the neurasthenic woman and her "anemia" is a large one, deserving of much more attention than it has been given. Were these women diagnosed and treated for what they really are, the sales of liver extract might be reduced (conservatively) some 75 per cent. The problem is one which really lies in the realm of the art rather than of the science of medicine—the art of treating a sick woman, not with needles, but with carefully chosen words and a reassuring frankness!

WILLIAM DAMESHEK, M.D.

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# ABSTRACTS

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## HEMATOPOIETIC TISSUES

DEVELOPMENT OF BONE MARROW IN ADULT ANIMALS. B. Steinberg and V. Hafford. From Toledo Hospital Institute of Medical Research. *Arch. Path.* 43: 117-126, 1947.

Previous studies of marrow regeneration by other investigators have centered around hypoplastic marrows induced by chemicals, starvation, or disease. In the present article, regeneration of the complete marrow was studied following its removal mechanically. The latter was accomplished by forcing sterile liquid petrolatum and saline through rabbit tibiae after holes had been drilled at appropriate locations. Forty-four rabbits were treated in this manner and one or more of the animals were killed at intervals of 1 to 60 days. Regeneration was initiated during the first 9 days by the endosteum sending out sheets of primitive reticular cells. Bony trabeculae were also laid down. Fat spaces were formed by the coalescence of vacuoles in two or more adjacent reticular cells. Between 12 and 17 days islands of myeloid cells were seen throughout the re-forming marrow. By the twenty-first day the marrow cavity was unevenly filled and the bony trabeculae had disappeared. These studies indicate that regeneration of all myeloid elements starts from a primitive reticular cell.

O. P. J.

## ERYTHROCYTES AND ERYTHROCYTIC DISEASE

COMPARISON OF THE EFFECTS OF MASSIVE BLOOD TRANSFUSIONS AND OF LIVER EXTRACT IN PERNICIOUS ANEMIA. C. S. Davidson, J. C. Murphy, R. J. Watson, and W. B. Castle. From the Thorndike Memorial Laboratory, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass. *J. Clin. Investigation* 25: 858-869, 1946.

The authors describe the clinical and hemarologic responses obtained in 5 patients with typical Addisonian pernicious anemia who received liver extract after the anemia had been partially or completely corrected by means of whole blood or red cell transfusions. It was found that very little symptomatic improvement was effected by transfusion, excepting for the relief of those symptoms specifically attributable to hemoglobin deficiency; anorexia, apathy, and digestive symptoms persisted until liver therapy was instituted. Leukopenia and thrombopenia were completely unaffected, regardless of the amount of blood given or the level of hemoglobin concentration attained. Following the subsequent course of liver injections, however, the white cell and platelet counts promptly returned to normal. Reticulocytosis, in response to liver extract, was reduced or absent in these transfused patients, depending upon the presence and degree of the anemia existing at the time liver therapy was instituted. Transfusions alone did not stimulate a reticulocyte response. Serial examinations of the bone marrow of these patients indicated that megaloblastic proliferation was promptly reduced as a result of artificial blood replacement. When complete correction of the anemia had been accomplished by transfusion, the appearance of the marrow was relatively normal. Later, as the donor red cells were eliminated and anemia recurred, the marrow again assumed a megaloblastic character.

The megaloblastosis of uncompensated pernicious anemia is the expression of a specific deficiency

state, and can be eliminated by administration of the maturation factor, or factors, present in liver extract. It now appears that the phenomenon requires, in addition, the erythropoietic stimulus of bone marrow hypoxia, a product of the anemia. Alleviating this hypoxia, by artificially increasing the concentration of circulating red cells, caused a reduction of megaloblasts, although the fundamental deficiency remained uncorrected. The data presented are, moreover, adequate to refute the hypothesis (Clinics 5: 708-726, 1946) that the neutropenia and thrombopenia, characteristic of pernicious anemia in relapse, are on a myelophthisic basis, attributable to megaloblastic proliferation in the marrow. On the contrary, it may justifiably be assumed that the normal development and survival of granulocytes and platelets, as well as of red cells, are dependent upon a common nutrient factor which is supplied in liver extract.

C. P. E.

RELATIVE CLINICAL AND HEMATOLOGIC EFFECTS OF CONCENTRATED LIVER EXTRACT, SYNTHETIC FOLIC ACID AND SYNTHETIC 5-METHYL URACIL IN THE TREATMENT OF MACROCYTIC ANEMIAS IN RELAPSE. W. B. Frommeyer and T. D. Spies. From Hillman Hospital, Birmingham, Alabama. Am. J. M. Sc. 213: 135-149, 1947.

The authors report the results of studying 100 patients treated with concentrated liver extract, 24 patients treated with synthetic folic acid, and 14 patients treated with synthetic 5-methyl uracil. Each of these three substances caused a remission. Although the clinical and hematologic response to folic acid paralleled that following concentrated liver extract therapy, the latter produced a greater rate of regeneration. Synthetic 5-methyl uracil was the least effective of the three substances.

O. P. J.

FOLIC ACID IN PERNICIOUS ANEMIA. FAILURE TO PREVENT NEUROLOGIC RELAPSE. R. W. Heinle and A. D. Welch. From the Departments of Medicine and Pharmacology, Western Reserve University College of Medicine, and the Lakeside Hospital, Cleveland. J. A. M. A. 133: 739-741, 1947.

The substitution of folic acid for liver extract in the therapy of pernicious anemia has awaited evaluation of the status of neurologic changes under the new form of treatment. Reports are now forthcoming which suggest that folic acid does not necessarily prevent neurologic progress in pernicious anemia, and make the routine use of this substance alone at present undesirable.

Heinle and Welch report that of 47 patients with pernicious anemia treated with folic acid, 3 had neurologic relapses (6.4 per cent). Two relapses were mild and responded readily to treatment with liver extract. The third patient, who forms the basis for the present report, developed an "explosive" neurologic relapse three months after the initiation of folic acid therapy, at a time when the blood status was normal. Response to further treatment did not occur for one month after intensive therapy with crude and refined liver extract and vitamin B was started.

The exact nature of the neurologic lesion described is not clear. It is certainly not the characteristic posterolateral sclerosis of pernicious anemia, but resembles rather a peripheral neuropathy. It began suddenly with numbness in the hands and was followed by numbness in the forearms and, later, feet, legs, and hips. All the deep tendon reflexes were absent; there was no vibratory sensation below the ribs (before therapy, this was normal); and there were no pathologic reflexes. Improvement was delayed and slow. That this could be peripheral neuropathy due to pernicious anemia is certainly possible.

This patient, as well as the other 2 with neurologic relapse, had very poor dietary intake. The authors speculate upon the relationship of this malnutrition to the occurrence of the relapses. The question as to neuropathy due to other causes (e.g., avitaminosis B) is not discussed. The authors believe that, in certain instances, folic acid may perhaps not only allow but even precipitate neurologic relapse in pernicious anemia. At any rate, liver extract remains the treatment of choice in pernicious anemia at this time.

S. E.

THE ANTI-ANEMIC PROPERTIES OF PTEROYLGLUTAMYL GLUTAMIC ACID. T. D. Spies, R. E. Stone, and R. Lopez Toca. From the Department of Medicine, University of Cincinnati College of Medicine, and the Hillman Hospital, Birmingham, Ala. South M. J. 40: 175-176, 1947.

Folic acid is pteroylglutamic acid and has one glutamic acid residue. A related compound, sythetic

peroxylglutamyl glutamic acid—containing two glutamic acid residues instead of one—was successfully utilized by the authors in treating 3 cases of macrocytic anemia (2 of pernicious anemia, 1 of nutritional macrocytic anemia). In a patient with pernicious anemia, an oral dose of 20 milligrams of this new substance daily for ten days resulted in a reticulocytosis of 25.1 per cent on the eighth day; an increase in red count from 1.4 M. to 2.18 M. and in hemoglobin from 5.2 grams to 6.7 grams by the tenth day; and clinical improvement.

Other synthetic products chemically related to folic acid may be expected to have similar effect in pernicious and related anemias. Evaluation of their efficiency and correlation of changes in chemical structure with degree of response may in time lead to better therapies and more fundamental knowledge of the nature of pernicious anemia. This report merely demonstrates the efficacy of the first such chemical modification of folic acid.

S. E.

THE ANEMIA OF INFECTION. V. FATE OF INJECTED RADIOACTIVE IRON IN THE PRESENCE OF INFLAMMATION. G. R. Greenberg, H. Ashenbrucker, M. Lauritsen, W. Worth, S. R. Humphreys, and M. M. Winthrope. From the Department of Medicine, University of Utah Medical School, Salt Lake City, Utah. *J. Clin. Investigation* 27: 121-125, 1947.

The experiments reported in this communication were designed to ascertain the mechanism by which iron metabolism is altered in the presence of inflammatory lesions, as manifested by the occurrence of hypoferrernia and the development of hypochromic anemia. As described elsewhere (*J. Clin. Investigation* 25: 65, 1946; 26: 114, 1947) these authors had determined that, whereas iron absorption is adequate in patients with chronic infection and anemia, the utilization of iron for hemoglobin production is sharply reduced, depending on the severity of infection. Iron administered by injection rapidly disappeared from the plasma. This diversion could not be attributed to excretory loss and was clearly not the result of erythropoietic activity, relatively little being incorporated into hemoglobin until the infection had been relieved.

The present report described experiments in which a radioactive isotope of iron ( $\text{Fe}^{59}$ ) was administered parenterally into normal rats, and rats with acute inflammatory lesions produced by the intramuscular injection of turpentine or bacterial cultures. The animals were subsequently sacrificed and radioactivity measurements carried out on isolated tissues. It was found that, in the presence of inflammation, the major accumulation of iron was in the liver, normally the chief storage site for this element; a relatively lower proportion of the material had been incorporated into red cells in comparison with the control animals. Most of the infected  $\text{Fe}^{59}$  could be accounted for in the liver and blood, very little being detected elsewhere, including the inflamed tissues.

A preferential diversion of iron to the storage depots, mainly the liver, may therefore be responsible for the hypoferrernia associated with infection. The relationship of this phenomenon to the development of anemia is, however, still obscure, for the failure of iron utilization, in the face of ample stores of potentially available iron, remains to be explained. Other metabolic faults, as indicated by the authors, may be implicated, for example a disturbance of protein metabolism involving the protein moiety of hemoglobin.

C. P. E.

ATYPICAL ANEMIA, WITH SPHEROCYTES AND TARGET CELLS COEXISTING IN THE BLOOD. G. Dintchev and G. Watkinson. From the Department of Pathology, St. Bartholomew's Hospital, London. *Am. J. M. Sc.* 213: 143-159, 1947.

The authors present a very interesting case which may represent either a new syndrome or an unusual response to some stimulus. The peripheral blood contained both spherocytes and target cells, so that lysis of red cells was just appreciable in 0.6 per cent saline, half complete in 0.335 per cent saline, and incomplete in distilled water. Studies of bone marrow preparations revealed many cytologic abnormalities. Granulocytic precursors showed a nucleic acid starvation and the formation of tetraploid giant cells. Among the erythroblasts there was a breakdown of normal spindle development and a replacement by abnormal multipolar spindles. During anaphase there was an incomplete separation. Some cells contained micronuclei derived from chromosomes dissociated from spindles which failed to reconstitute daughter nuclei. Hypoploid mitoses with as few as a dozen chromosomes were present and some

of these were in tripolar mitosis. The marrow differed from pernicious anemia in that it was normoblastic. Anatomic diagnosis at autopsy was: pneumonia (virus type), cortical necrosis of kidney, hyperplasia of bone marrow with myeloid metaplasia in liver, spleen, and renal pelvic fat.

O. P. J.

WEBS AND CONSTRICTING BANDS IN THE UPPER ESOPHAGUS (SIDEROPENIC DYSPHAGIA). *M. A. Thomas*. Radiological Group, Cleveland, Ohio. *Am. J. Roentgenol.* 57: 213-219, 1947.

Four cases of dysphagia due to upper esophageal lesions are reported. In 3, there were thin membranes just below the level of the cricoid cartilage, and in the fourth, an esophageal stricture. The authors point out that the lesion is frequently missed because of its high location. Capsules filled with barium were swallowed and became lodged just above the membrane. The bands were cut with an esophagoscope with relief of symptoms. Three patients had a significant degree of anemia. The lesions were similar in appearance and location to those reported in a more extensive article by Waldenstrom and Kyellberg. Both authors felt that the lesion was a manifestation of iron deficiency and provided the anatomic explanation for the dysphagia of the Plummer-Vinson syndrome.

C. A. F.

SUR UN CARACTÈRE ESSENTIEL DE L'ANÉMIE PERNICIEUSE (ON AN ESSENTIAL FEATURE OF Pernicious Anemia). *M. Lourtau*. From the Institut de Biologie physicochimique, Paris. *Le sang.* 4: 242-246, 1946.

TITRAGE DU FACTEUR ANTIPERNICIEUX PAR UNE MÉTHODE BIOLOGIQUE (TITRATION OF THE ANTI-PERNICIOUS FACTOR BY A BIOLOGICAL METHOD). *M. Lourtau*. *Le sang.* 6: 363-375, 1946.

SUR LA DISPARITION DU FACTEUR ANTIPERNICIEUX DANS LE SATURNISME EXPÉRIMENTAL (ON THE DISAPPEARANCE OF THE ANTI-PERNICIOUS FACTOR IN EXPERIMENTAL SATURNISM). *M. Lourtau*. *Le sang.* 8: 517-523, 1946.

We have collected these three articles concerning a study of the relations between experimental saturnine anemia of the rabbit and human pernicious anemia. According to the author, anemia is easily obtained by intravenous injection of a water solution of neutral lead acetate at a concentration 3.63 Gm. per liter, this anemia has the following features: first hypochromic anemia, then hyperchromic anemia, the globular value being increased; the bone marrow shows a normoblastic activity. Even when the anemia is cured, the high hemoglobin concentration persists for some time. Oral administration of lead was found to be more effective than injections, and the author gave 2 cc. of a 6.5 per cent solution every other day during 15 days. When liver extract was given to these anemic animals, there was a fall of the corpuscular volume, the intensity and the speed of which was quite specific. In the titration of liver extract one attempts to find the threshold of activity, and thus to determine the minimum dose which is active. This was the same for all the animals, was independent of the experimental conditions, and was called the "rabbit unit." The most important fact was the sudden fall of the globular value (in the hyperchromic anemias as well as in the other types of anemia). This was more striking when the globular value was very high, but it was always present when the globular value was above 0.14 per cent (mean value is from 0.16 to 0.44 per cent, and it was far higher than any spontaneous fall observed). Each extract ought to be titrated on at least 3 rabbits. The antipernicious titration of untreated rabbit's liver is quite constant, provided that the antipernicious activity is related to 1 gram of fresh liver extract (the globular concentration varying with the liver weight). The saturnine intoxication is associated with a complete disappearance of the antipernicious factor which is persistent if not permanent.

Thus, a new biologic titration method of the antipernicious liver extract is proposed. Although the data concerning the titration of the hemoglobin are somewhat inadequate and although this experimental anemia is normoblastic and not megaloblastic as in man, the importance of any new method of titration is worth verification by more extended investigations (M. C. V., M. C. H., M. C. H. C., which are more precise than the globular value).

J. P. S.

ÉTUDES SUR L'ANÉMIE EXPÉRIMENTALE PAR INANITION PROTÉIQUE CHEZ LE RAT, LES ALTÉRATIONS DU SANG DE LA MOËLLE OSSUEUSE ET DE LA RATE, LES RÉLATIONS AVEC LE CYCLE OESTRAL (Study on the Experimental Anemia Produced by Protein Inanition in Rats. Blood, Bone Marrow and Spleen Modifications and Their Relation to the Evolution of the Estrous Cycle). *A. Aschkenazy*. From the Institut de physiologie générale des Facultés de Strasbourg et de Lyon. *Le sang.* 1: 34-61, 1946.

Ten white rats were fed a protein-free diet; they showed the following symptoms: a fall of the hemoglobin beginning on the 21st day; a severe anemia by the 38th day, after a decrease of 34 per cent in weight, a few normoblasts at first and then more numerous normoblasts in very anemic rats, a leukopenia, with neutropenia, and an alteration in the lymphocytes.

There was a normoblastic hyperplasia of the bone marrow, and a splenic atrophy, which was much more frequent than liver atrophy (65.5 per cent). There was some alteration of the estrous cycle (irregularity, then interruption) earlier than the blood injury.

There are 60 bibliographic references.

J. P. S.

# LA MÉTHIONINE DANS LE TRAITEMENT DES ANÉMIES (METHIONINE IN THE TREATMENT OF ANEMIAS).

A. Gadjos. Travail de la clinique médicale de l'Hôtel Dieu; Pr. H. Bénard—Faculté de Médecine de l'Université de Paris. *Revue d'hématologie* 1: 117-141, 1942.

The author begins with an historical review of methionine, its activity and its metabolism. He made the following experiments: Every day for 17 days carbon-tetrachloride was injected subcutaneously into 9 rats; the dose was increased from 0.05 cc. to 0.9 cc. and the rats were fed only on wheat. Three of these rats were kept as controls; the others were injected with 15 to 30 mg. of methionine each day. In spite of the well-known activity of methionine, the rats did not appear to be protected against liver injury: they became icteric about the fourth or fifth day. The histological examination showed a marked fatty degeneration of the liver, the same as in the controls, and an identical amount of lipids in the liver. On the other hand, erythropoiesis was quite different in the two series: the control rats had a marked anemia, but there was no anemia in the rats treated with methionine. Another experiment (8 rats) gave the same results. In a third experiment, 10 rats were given a cirrhotogenic diet rich in lipids and low in proteins, plus 100 mg. of L-cystine a day. In this experiment the protective activity of methionine on the liver was evident, the activity on erythropoiesis still frank, only the controls were anemic after the third month. In man, the author gave 2 mg. of methionine each day for 2 to 4 weeks to 10 cases of anemia of the following types: 4 severe macrocytic anemias, 2 pernicious anemias, 4 normochromic normocytic anemias. The results were as follows: no improvement in the pernicious anemias; but in 7 out of the 8 other cases there was a notable or striking improvement: a moderate increase of the reticulocytes about the fifth day (4 to 8 per cent), an average gain of 1,100,000 red cells in two weeks. This red cell increase was accompanied with an abatement of the serum iron and serum copper.

J. P. S.

# APPLICATION OF THE RADIOACTIVE RED CELL METHOD FOR DETERMINATION OF BLOOD VOLUME IN HUMANS.

G. R. Mentely, E. B. Wells, and P. F. Hahn. From the Departments of Medicine and Biochemistry, Vanderbilt University School of Medicine. *Am. J. Physiol.* 148: 531-537, 1947.

In addition to other uses, radioactive iron promises to provide a direct method of measuring the volume of the blood, in contrast to the present dye methods, all of which are indirect. The method depends upon the injection of a known amount of radioactivity, and the subsequent determination (a few minutes later) of the distribution of the radioactivity in the blood of the recipient. Since all the radioactive iron is lodged within red cells, the mass of red cells can thus be calculated directly, and no correction for a "mixing phase" is necessary.

The authors gave  $\text{Fe}^{59}$  to volunteer, recently phlebotomized normal individuals, until their blood had a radioactivity of 1000 counts per minute per ml. of blood. This level was maintained by booster doses as required. Freshly drawn citrated radioactive blood was then injected into patients whose blood volume was to be determined; and, for purposes of comparison, Evans blue was simultaneously injected into the same individuals. After 15 minutes to allow for mixing, successive samples of blood were drawn for determination of the concentration of the dye (serum) and for determination of radioactivity (whole blood). The usual method of Gihson and Evans was used to determine plasma volume and, by means of hematocrit, corresponding whole blood volume. Aliquots of the red cell samples were treated for determination of radioactivity, and the red cell mass thus determined; the hematocrit was then used to determine the total blood volume.

It was found that the blood volume as determined by the direct radioactivity method was 81 per cent, on the average, of that by the Evans blue method. The direct value was presumably the more accurate.

result, since an error of unknown magnitude is introduced during the mixing phase of the Evans blue by phagocytosis of the dye by reticulo-endothelial cells.

This paper confirms the existence of the discrepancy between the two types of determination of blood volume. The authors justifiably recommend caution in the use of indirect methods for this determination. S. E.

## HEMOGLOBIN, HEMOGLOBINURIA, AND BLOOD PIGMENT METABOLISM

PLASMA PROTEIN AND HEMOGLOBIN PRODUCTION. DELETION OF INDIVIDUAL AMINO ACIDS FROM GROWTH MIXTURE OF TEN ESSENTIAL AMINO ACIDS. SIGNIFICANT CHANGES IN URINARY NITROGEN. F. S. Robscheit-Robbins, L. L. Miller, and G. H. Whipple. From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y. *J. Exper. Med.* 85: 243-265, 1947.

This paper and the following two papers are concerned with studies on dogs with "double depletion" (anemia and hypoproteinemia) produced by blood removal and a low or nonprotein diet plus abundant iron. Blood protein output and urinary nitrogen balance were measured in such dogs after feeding standard growth mixtures of essential amino acids and mixtures lacking one of the essential amino acids. It was found that tryptophane and to a less extent phenylalanine and threonine when returned to the amino acid mixture were associated with a preponderance of plasma protein output, while arginine, lysine, and histidine were associated with a preponderance of hemoglobin output.

The average figure for production ratio (protein output to intake) was 25 per cent when an essential amino acid was deleted from the complete mixture, 19 per cent when the complete amino acid mixture was administered, and 15 per cent when a good diet protein was fed. It is suggested that, when given whole protein, the severely depleted dog demonstrates a flow of protein-forming materials to organ tissues to replete these stores of protein before the production of new hemoglobin and plasma protein begins. It is further suggested that from tissue protein lost on feeding amino acid mixtures come materials which accelerate production of hemoglobin and plasma protein in depleted dogs. The amino acid mixtures are conserved and the urinary nitrogen shows a positive balance. Some of this amino acid material probably goes into new-formed blood proteins, and "raiding" of body protein stores probably comes into this reaction as the intake of protein or amino acid decreases.

L. E. Y.

ANEMIA AND HYPOPROTEINEMIA. WEIGHT MAINTENANCE EFFECTED BY FOOD PROTEINS BUT NOT BY MIXTURES OF PURE AMINO ACIDS. L. L. Miller, F. S. Robscheit-Robbins, and G. H. Whipple. From the Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y. *J. Exper. Med.* 85: 267-275, 1947.

The authors showed that in doubly depleted dogs good food proteins in adequate amounts maintain body weight, a strongly positive nitrogen balance, and produce considerable amounts of new hemoglobin and plasma protein. Under comparable conditions mixtures of the pure amino acids essential for growth produce large amounts of new hemoglobin and plasma protein and a positive nitrogen balance but do not maintain body weight. It is concluded that some unidentified substance or compound present in certain proteins but absent in mixtures of essential amino acids may be responsible for the differences in the response of the doubly depleted dog.

L. E. Y.

RAIDING OF BODY TISSUE PROTEIN TO FORM PLASMA PROTEIN AND HEMOGLOBIN. WHAT IS PREMORTAL RISE OF URINARY NITROGEN? G. H. Whipple, L. L. Miller, and F. S. Robscheit-Robbins. From Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y. *J. Exper. Med.* 85: 277-286, 1947.

In this portion of the study on doubly depleted dogs the authors report a blood protein production of from 50 to 140 grams for every kilogram of weight loss. Body and tissue protein are raided to fill the demand for new hemoglobin and plasma protein—an illustration of the ebb and flow or dynamic equilibrium between organ or tissue protein and blood proteins, with the latter having priority. The largest blood protein output is observed when weight loss is most rapid. A premortal rise in urinary nitrogen was

not observed in these studies. It is suggested that this phenomenon, which has been reported in long-term fasting experiments by other investigators, may be due to terminal infection.

L. E. Y.

**TREATMENT OF PAROXYSMAL HEMOGLOBINURIA WITH PENICILLIN. REPORT OF A CASE.** L. C. Goldberg. From Oliver General Hospital, Augusta, Georgia. *Am. J. Syph., Gon., & Ven. Dis.* 31: 163-165, 1947.

It is generally stated that no treatment exists for the paroxysmal hemoglobinuria associated with tertiary syphilis, but that treatment of the syphilis may be beneficial. The effects of such therapy remain rather dubious. In the present note, the author reports the use of penicillin for this purpose.

The patient was a 26 year old soldier who had 100 episodes of chills, fever, abdominal pain, headache, and dark urine—always after exposure to cold—in one year. There were no history or physical findings of syphilis, but the Wassermann and Kahn tests were positive, and a Donath-Landsteiner hemolysin was demonstrated in the serum. Immersion of hands or feet into cool water invariably produced hemoglobinuria.

Since treatment with bismuth had already been employed without effect, penicillin was tried, 3.6 million units being given in two days. During the following month, there were only two paroxysms, and these were mild and not associated with systemic symptoms. This was an unusually small number of episodes for the patient. Cooling of the extremities, however, still resulted in hemoglobinuria, although without constitutional symptoms.

As the author points out, no definite conclusions can be drawn from this incomplete case. It is probably doubtful if the fundametal serum abnormality can be affected by antibiotic therapy.

S. E.

**A STUDY OF THE MECHANISM OF THE METHYLENE BLUE TEST FOR BILE PIGMENT IN URINE. PREPARATION OF A COMPOUND OF METHYLENE BLUE AND BILIRUBIN.** J. G. Reinbold and C. B. Fowler. From the University of Pennsylvania Nutrition Service and Biochemical Laboratory of the Philadelphia General Hospital, Philadelphia, Pa. *J. Biol. Chem.*, 167: 401-406, 1947.

This paper would appear to explain the disagreement over the methylene blue test. In alkaline solutions, bilirubin was found to react one equivalent of bilirubin to two equivalents of methylene blue to form a green compound, the spectral characteristics of which resemble closely the mixture of the reacting pigments. However, the pH of urine is seldom alkaline enough to bring about this reaction. The authors concluded that the methylene blue test for bilirubin in the urine depended primarily on color blending.

C. A. F.

## BLOOD GROUPS, THE Rh FACTOR BLOOD TRANSFUSION

**THE Rh BLOOD GROUPS.** R. R. Race. *Schweiz. med. Wchnschr.* 76: 921-925, 1946.

This paper, which is written in English, reviews briefly the contributions of Levine, Landsteiner, and Wiener in initiating study of the Rh blood groups and then concerns itself with the details of R. A. Fisher's theory of the composition of the Rh-chromosome and the inheritance of the Rh-genes. Fisher's theory postulates three loci on the Rh chromosome, each locus having two allelomorphs. The nomenclature and its equivalent in the usual American terminology are as follows:

$Rh_0 = D$ ; allelomorph is  $Hr_0 = d$

$Rh' = C$ ; allelomorph is  $Hr' = c$

$Rh'' = E$ ; allelomorph is  $Hr'' = e$

Two chromosomes, each with its three loci, determine the Rh status (genotype) of an individual. Any individual's Rh status, correspondingly, can (and must) be represented by six allelomorphic genes. For example, an  $Rh_0 Rh'$  individual is represented by  $cDe/cde$ ; an  $Rh_0 Rh''$  individual is represented by  $cDe/cde$ ; etc. Within the gene, Rh and Hr are completely reciprocal, and at each locus one or the other (but not both) is present. [I.e., an  $Rh$  gene consists of  $D$  or  $Hr'$ ,  $Rh_0$   $Hr''$ , and reacts with antiRh serum, antiHr serum, and antiHr' serum.] In actual practice, of course, the formula of the genotype (i.e., of the two chromosomes) is not always subject to discovery with the tools at hand today.

Fisher's theory allows easy comprehension of two recent subgroups of the Rh factor. The first, labeled  $C^w$ , is a subgroup of C ( $Rh'$ ) and occurs instead of C or c at the same chromosome locus. The second, called  $D^w$ , is a subgroup of D ( $Rh_0$ ) and occurs at the D-d locus instead of the more usual D or d. Both

these subgroups have recently been discovered in England, and the irregular reactions provoked by their presence in certain individuals have been made comprehensible by Fisher's simple picture of the Rh chromosome.

This short article by Dr. Race summarizes excellently the fundamentals of Rh knowledge as they are known today. The reasonableness of Fisher's postulates is confirmed by the statistical data presented. The use of the Fisher terminology commendably simplifies the discussion of the "Rh" problem.

S. E.

**SUBSTITUTION TRANSFUSION: A NEW TREATMENT FOR SEVERE ERYTHROBLASTOSIS FETALIS.** *H. Wallerstein.*

From the Department of Hematology, Jewish Memorial Hospital, New York City, and the Laboratories of the Queens General Hospital, Jamaica, N. Y. *Am. J. Dis. Child.* 73: 19-33, 1947.

The procedure described is that of removing the infant's blood from the longitudinal sinus through the anterior fontanel and replacing it with Rh negative blood given through a cannula inserted in a superficial vein of the arm. After about 50 to 60 cc. of blood have been removed by syringe, an equal quantity of Rh negative blood is administered. When this type of exchange has been repeated at least five times, the needle is removed from the fontanel and the infusion in the arm is continued until about 75 to 100 cc. of blood are given in excess of the amount removed. At the end of this procedure it can be shown by differential agglutination that only 20 to 25 per cent of the infant's original Rh positive cells remain.

The objectives of substitution transfusions are to remove the products of hemolysis and to prevent their formation in excessive amounts by the removal of the Rh positive erythrocytes before they are destroyed in large numbers by the maternal antibody. In this way it is hoped that the incidence of hepatic damage and kernicterus can be minimized. Seven of the 9 patients cited in this report responded satisfactorily, and it is the author's opinion that the 2 infants who died might have recovered if treatment could have been instituted during the first 24 hours of life.

Substitution transfusion is recommended chiefly as a prophylactic procedure to be applied during the subicteric period immediately after birth. History of erythroblastosis in a previous infant is emphasized as the principal indication for its use, but due consideration is also given to serologic data, especially a rising titer of maternal antibody.

The results of substitution transfusion thus far reported by a number of investigators are encouraging, but there is an obvious need for further critical evaluation of the efficacy and indications for this procedure. The technic described by the author is relatively simple and, in experienced hands, this form of therapy should be safe.

L. E. Y.

**THERAPY OF ERYTHROBLASTOSIS FETALIS WITH EXCHANGE TRANSFUSION.** *A. S. Wiener, I. B. Wexler, and T. H. Grundfast.*

From the Transfusion Division and the Departments of Pediatrics and of Gynecology of the Jewish Hospital of Brooklyn. *Bull. New York Acad. Med.* 23: 207-220, 1947.

The technic described involves injection of the donor's Rh negative blood into the saphenous vein at the ankle, withdrawal of the infant's blood through the radial artery at the wrist, and the use of heparin to prevent clotting of the infant's blood. The transfusion is kept about 50 to 75 cc. ahead of the bleeding in order to allow a margin of safety, but the total amount injected is not allowed to exceed by more than 50 cc. the quantity of blood withdrawn. The modified technic recommended also calls for replacement of one half the donor's plasma with saline in order to minimize "conglutination" of the Rh positive cells remaining in the infant's circulation.

Of a total of 17 infants treated by this method, many of them critically ill, all but 1 made a prompt recovery. Details are given concerning 2 cases, in both of which a 90 per cent replacement was effected. In 1 case the maternal serum contained univalent Rh antibodies in moderate titer, and in the other bivalent antibodies were present in high titer. In the former case Rh positive cells were absent in the infant's circulation from the 7th to the 34th days, and in the latter from the 5th to the 15th days. The authors explain the shorter period of "ineffectual regeneration" of erythrocytes in the second case on the hypothesis that bivalent antibodies enter the infant's circulation mainly during labor and are largely removed by exchange transfusion. Univalent antibodies, on the other hand, are thought to enter the infant's circulation continuously during the latter part of pregnancy and to permeate tissue fluids in such

a way that they are not readily removed by exchange transfusion. It will be of interest to see if this hypothesis can be substantiated by further observations following exchange transfusions.

L. E. Y.

ÉTUDES STATISTIQUES (CLINIQUES ET SÉROLOGIQUES) SUR 50 FAMILLES ATTEINTES DE MALADIE HÉMO-  
LYTIC DU NOUVEAU-NÉ (STATISTICAL STUDIES, CLINICAL AND SEROLOGIC, ON 50 FAMILIES WITH HEMO-  
LYTIC DISEASE OF THE NEWBORN). M. Bessis. Travail du Centre National de Recherches Hématologiques  
et de Transfusion Sanguine (Hôpital St. Antoine, Paris). *Revue d'Hématologie* 1:167-220, 1946.

The author studied 225 gestations in 50 families; each case is illustrated by a chart. The statistical results are the following: 30 per cent of the children were uninjured (first-born or born of a heterozygous father). Of the 70 per cent injured: 10 per cent anemic, 40 per cent icterus gravis, 50 per cent born dead or abortive. Of those born dead, 41 per cent showed hydrops foetalis. Of those with icterus gravis, 13 per cent showed evidence of nuclear icterus. When the first-born child was affected, one could almost always find a previous sensitization of the mother by blood transfusion or heterohemotherapy. The study of the mortality in the various forms shows: hydrops foetalis 100 per cent; icterus gravis in general 71 per cent (but the percentage of death varies with the treatment: 96 per cent for the untreated cases, 57 per cent if treated with blood transfusion of unknown Rh type, 37 per cent if treated with transfusion of compatible Rh blood). Cases which were only anemic: no deaths. Four cases of cirrhosis of the liver and 1 case of sclerocystic degeneration of the kidneys were observed. The serologic data are the following: 92 per cent of the cases are due to an Rh<sub>0</sub> or Rh' incompatibility; 4 per cent to an Rh" incompatibility; 4 per cent to an H<sub>1</sub>' incompatibility. In these 50 families, 94 per cent showed an A.B.O. incompatibility (much more frequent than is normal). In 82 per cent of the cases an anti-Rh antibody was found in the mother's serum. This antibody was anti-Rh<sub>0</sub> in 80 per cent, anti-Rh' in 14 per cent, anti-Rh" in 4 per cent, anti-H<sub>1</sub>' in 2 per cent; it was an incomplete antibody in 15 per cent of these cases. In 2 cases the antigen appeared only after a biologic reactivation by small injections of Rh blood. Between the 22nd and the 30th days after the end of the pregnancy, the antibody was found in 93 per cent. This number falls to 83 per cent from one month to one year, and to 33 per cent after one year. Five times in 8 examinations, agglutinins were found in the milk.

J. P. S.

L'ANTIGÈNE O DANS SES RAPPORTS AVEC LA GÉNÉTIQUE ET LA SÉROLOGIE (THE ANTIGEN O, ITS RELATIONS  
WITH THE GENETIC AND THE SEROLOGY.) P. Moreau. *Revue d'Hématologie* 1: 266-267, 1946.

The author makes the following statements on the basis of his own experiments and publications on the subject. The antigen O is not specifically human but is heterogenic. It bears no relation to the O (recessive) gene. It is diversely distributed in the various red cells O, A<sub>1</sub>, A<sub>2</sub>, B, AB, and A<sub>2</sub>B. The differentiation between homo- and heterozygous A or B is still impossible. The anti-O agglutinin cannot appear in human plasma except in very rare circumstances. Practically, the study of antigen O has opened new possibilities for a better diagnosis of the subgroups A and for the control of universal donors. It is also useful for the study of some transfusion accidents and has some medicolegal applications. For all these reasons the antigen O is not without theoretical and practical interest.

J. P. S.

INFECTIOUS HEPATITIS IN RELATION TO BLOOD TRANSFUSION. P. E. Sartwell. *Bull. U. S. Army M. Dept.*, 7: 90-100, 1947.

By questionnaire, a survey was made of 1762 patients with hepatitis in sixty-four general army hospitals. Five hundred of these patients had received either blood or plasma at the time of their battle injury. In the nontransfused patients, the interval between injury and development of hepatitis showed a flat line with no peak incidence. Those transfused, however, showed a clear-cut peak at ten to fourteen weeks after injury (when presumably the blood was given). From the data presented, one might estimate that in about two thirds to three fourths of the transfused patients, the hepatitis could be ascribed to iatrogenic materials, presumably blood, received at the time of injury. This is further evidence of the importance of blood products as a cause of hepatitis.

C. A. F.

## LEUKEMIA AND LYMPHOMA

LYMPHOMAS AND LEUKEMIAS. L. F. Crater. From the Memorial Hospital, New York, N. Y. Bull. New York Acad. Med. 23: 79-100, 1947.

The author presents an excellent appraisal of current knowledge of lymphomas and leukemias, with particular reference to diagnosis, prognosis, therapy, and relationship to the general problem of malignant diseases. It is emphasized that tumors of the lymphatics and blood-forming organs are important because (1) they are collectively responsible for over 6 per cent of all deaths from malignancy, (2) they strike the younger elements of the population, (3) their range of morbidity brings them within the scope of every kind of practitioner, and (4) they lend themselves well to clinical investigation of cancer and trial of new therapeutic methods. The author stresses the fact that proper therapy may be not only palliative but also life-prolonging, and he sees some promise of curability in certain types of Hodgkin's disease and lymphosarcoma.

L. E. Y.

PARTIAL MATURATION OF LEUKEMIC MYELOBLASTS FOLLOWING FRESH PLASMA TRANSFUSIONS. J. L. Schwind. From the Department of Anatomy, University of Cincinnati. Am. J. M. Sc. 213: 170-175, 1947.

After first redefining the myeloblast and myelocyte A as seen in supravital preparations, Schwind very carefully studied the changes in leukemic blood from 2 patients following various transfusions. Fresh normal blood plasma produced a partial maturing effect on the myeloblasts. The causative substance was not present in gamma globulin or in dried plasma.

O. P. J.

EFFECTS OF RADIOACTIVE PHOSPHORUS ( $P^{32}$ ) ON NORMAL TISSUES. W. R. Platt. From the Department of Pathology, Washington University School of Medicine, St. Louis. Arch. Path. 43: 1-14, 1947.

Changes produced by the radioactive isotope of phosphorus ( $P^{32}$ ) were studied in tissues from 43 cases, the diagnoses of which included acute leukemia, chronic leukemia, leukosarcoma, aleukemic leukemia, Hodgkin's disease, multiple myeloma, lymphosarcoma, melanoma, and Ewing's sarcoma. Radioactivity of bone marrow, liver, spleen, kidney, muscle, and lymph nodes was determined. Tissues which utilize phosphorus rapidly have a high content and take up higher concentrations of radioactive phosphorus. Normal as well as diseased tissues are affected. Radiophosphorus has a marked sclerosing effect on the bone marrow. Of the various immature marrow cells, megakaryocytes were the most sensitive—to the extent that they were either degenerated or completely absent.

O. P. J.

THE CHANGING CONCEPT OF MYELOMA OF BONE. E. Atgarter and R. Robbins. From the Departments of Pathology and Radiology, Temple University Medical School, Philadelphia, Pa. Am. J. M. Sc. 213: 282-289, 1947.

In recent years there has been a tendency to modify the concept of myeloma. Three clinical subtypes of this disease have been suggested. The classical type is a multicentric tumor involving the red marrow of flat bones. The other types have either a single focus which eventually becomes multiple or a diffuse involvement simultaneously of all red marrow. The present authors recognize two cytomorphic types of myeloma: one the plasma cell type and the other a myeloid type. The origin of these tumor cells may be from the reticulo-endothelial system. In 5 of the 13 cases, aspirated sternal marrow was examined. In 1 case no myeloma cells were encountered. In the other cases tumor plasma cells were present, even though 2 cases were diagnosed on sectioned tissue as the myeloid type and 2 as the plasma cell type.

O. P. J.

PRIMARY SYSTEMATIZED AMYLOIDOSIS WITH MACROGLOSSIA; A SYNDROME RELATED TO BENCE JONES PROTEINURIA AND MYELOMA. L. A. Brunsting and I. D. MacDonald. From the Mayo Clinic, Rochester, Minn. Proc. Staff Meet. Mayo Clin., 67-70, 1947.

Data on 4 cases are presented to show the interrelationship between primary "systematized" amyloidosis and plasmocytoma with Bence-Jones proteinuria. The patients had symptoms of muscle pain, dyspnea, weakness, and in 3 out of 4 there was macroglossia. Three cases gave a positive Congo red test

intradermally. In 3 patients there were atypical plasma cells in the marrow and Bence-Jones protein in the urine. X-rays, however, showed no bone lesion. In 2 autopsied cases there was distribution of amyloid as described in primary amyloidosis. The authors discuss the coincidence of primary amyloidosis and myeloma.

C. A. F.

CASE OF SARCOMA OF STOMACH SIMULATING SPLENIC TUMOUR. *B. Wolman*. From the Wichington Hospital, Manchester, England. *Brit. J. Surg.* 34: 322-323, 1947.

Sarcoma of the stomach is a rare tumor, comprising some 1 per cent of all gastric tumors (Ewing, 1940). The association of a macrocytic hyperchromic anemia with sarcoma is an even rarer occurrence. It is a coincidence that two reports of this combination should be published within a few months. The first was that of Schindler et al. (*Surg., Gynec., & Obst.* 82: 239-52, 1946), in which 1 patient with leiomyosarcoma of the stomach was first seen because of a typical picture of pernicious anemia, even including reticulocytosis on liver extract therapy. These authors wondered whether, in this case, pernicious anemia had antedated and led to sarcoma.

In the present paper, another instance of gastric sarcoma and "pernicious anemia" is reported. A woman aged 34 was found to have a tender tumor in the left mid-abdomen, which was thought to be spleen. Laboratory studies demonstrated a macrocytic hyperchromic anemia typical of pernicious anemia; gastric achlorhydria; and an increased number of megaloblasts in the bone marrow. There was, however, no response to liver therapy. At operation and subsequent autopsy, the tumor was found to be a spindle-cell sarcoma involving the stomach, omentum, mesentery, and peritoneum, and apparently primarily in the stomach. The spleen was normal.

The relationship between carcinoma of the stomach and pernicious anemia is well known. Carcinoma is prone to develop in a patient with pernicious anemia and, conversely, a macrocytic hyperchromic anemia may be the presenting complaint in carcinoma of the stomach. The common denominator is considered to be the atrophic gastric mucosa, a "precancerous" lesion. Since sarcoma of the stomach is a subepithelial lesion, and therefore not based upon the atrophic gastric mucosa, a relationship to macrocytic hyperchromic anemia would not be expected to occur. It is possible that some other type of mechanism must be postulated for this combination; or, perhaps, that the accepted mechanism for carcinoma needs modification. There is also the possibility that this combination is merely coincidental.

S. E.

THE USE OF NITROGEN MUSTARD IN THE TREATMENT OF LYMPHOMATA. *L. K. Alpert and S. S. Petersen*. *Bull.*

U. S. Army M. Dept. 7: 187-194, 1947.

Methyl bis beta-chloroethylamine hydrochloride was given in dosage of 0.1 mg. per kilo daily for four-day periods to 23 patients. These included 15 cases of Hodgkin's disease, 1 case of lymphosarcoma, 1 case of chronic myelogenous and 1 of chronic lymphocytic leukemia, 2 reticulum cell sarcomas, and 1 carcinoma in the lung. The only condition that responded satisfactorily was Hodgkin's disease. There were no serious complications of therapy. The average duration of remission appeared to be about two months. In this report there is nothing to suggest any superiority of this form of treatment over the expected response to x-ray.

C. A. F.

HISTOPLASMOSIS IN INFANCY. *M. Iams and H. M. Kiehl*. From the Mayo Clinic, Rochester, Minn. *J. Pediat.* 30: 123-128, 1947.

This report concerns the case of an infant with a fatal illness which was manifested by the progressive development of weight loss, fever, hepatosplenomegaly, lymphadenopathy, leukopenia, and a hypochromic, macrocytic anemia. A clinical diagnosis was established at age 7 months by the demonstration in a bone marrow biopsy specimen, of encapsulated inclusion bodies, 1-5 microns in diameter, each with a central dark chromatin mass. A few extracellular forms were discovered, but most were contained in mature neutrophils, eosinophiles, monocytes, and megakaryocytes. These findings are well illustrated by means of color plates. The fungus *Histoplasma capsulatum* was subsequently cultivated from the peripheral blood, duodenal aspirations, and stools, cultures being prepared on hormone blood agar plates containing added streptomycin.

Histoplasmosis, the authors emphasize, deserves serious consideration as a diagnostic possibility in cases with obscure fever and features suggesting a lymphoblastomatous disease.

C. P. E.

## AGRANULOCYTOSIS

AGRANULOCYTOSIS IN CHILDHOOD. REPORT OF A CASE WITH SERIAL BONE MARROW STUDIES. *E. Bruck*. From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio. *Am. J. Dis. Child.* 73: 186-194, 1947.

A well-studied case of agranulocytosis of unknown etiology in a 2 year old girl is presented. The child recovered despite the fact that the number of granulocytes in the blood varied between 0 and 84 per cu. mm. for 11 days and the illness was complicated by pneumonia and severe stomatitis. At the height of the disease, hypoplastic bone marrow with lymphocytic reaction was found. Although penicillin, blood transfusions, crude liver extract, and pentnucleotide were used in this case, the author stresses the now accepted fact that prevention and treatment of infection are of paramount importance in the management of agranulocytosis.

This is the second case of acute or Schultz type agranulocytosis in a child reported since 1937. All other cases in children reported during the past ten years were due either to sulfonamide compounds or to severe and protracted purulent infections. There were no children in Plum's series of 88 cases and he accepted only 9 cases of agranulocytosis in children reported in the literature up to 1937.

L. E. Y.

## BLOOD COAGULATION AND HEMORRHAGIC DISEASES

CHEMICAL, CLINICAL AND IMMUNOLOGIC STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION. XXXIII. THE COAGULATION DEFECT IN HEMOPHILIA: THE EFFECT IN VITRO AND IN VIVO ON THE COAGULATION TIME IN HEMOPHILIA OF A PROTHROMBIN AND FIBRINOGEN-FREE NORMAL PLASMA AND ITS DERIVED PROTEIN FRACTION. *J. H. Lewis, J. P. Souther, and F. H. L. Taylor*. From the Thorndike Memorial Laboratory, Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass. *J. Clin. Investigation*, 25: 876-879, 1946.

A material suitable for intravenous injection was prepared by heating a fraction of normal human plasma (Fraction I), in which the clotting factors are mainly concentrated. The resulting product, tested *in vitro* and *in vivo*, was found to have lost none of the antihemophilic potency characterizing Fraction I when unheated. Inasmuch as fibrinogen, prothrombin and all formed elements, including platelets, were completely removed by the heating process, further confirmation was obtained of the fact that these substances are not responsible for the clot-promoting properties of normal plasma when the latter is added to hemophilic blood, or when administered to patients with hemophilia. From the viewpoint of practical therapeutics, it is reasonable to infer that an improved substitution therapy for this disease may be in prospect, one safer, more convenient and efficient than whole blood or plasma transfusion.

C. P. E.

THE MECHANISM OF HEMOSTASIS. *L. M. Tocantins*. From the Division of Hematology, Department of Medicine, Jefferson Medical College, Philadelphia, Pa. *Ann. Surg.* 125: 292-310, 1947.

The author presents a well-illustrated, expository review and a coherent working concept of the mechanism of hemostasis. The extravascular, vascular, and intravascular factors concerned in checking blood loss are discussed concisely. A list is given of the probable order of importance of the components of hemostasis in different vessels, vascular contraction being rated most important in checking blood loss from arteries and arterioles, external compression in capillaries and venules, and platelet massing in veins. General deductions are that (a) the nature of the defect in hemostasis largely determines what vessels will be affected, (b) failure of spontaneous hemostasis may not necessarily follow a deficiency of one or two factors provided stresses are moderate and exerted chiefly on vessels not wholly dependent on those factors to check hemorrhage, and (c) most methods of arresting blood loss are attempts to duplicate the steps of the natural mechanism of hemostasis.

L. E. Y.

TREATMENT OF TWO REACTIONS DUE TO GOLD. RESPONSE OF THROMBOCYTOPENIA AND GRANULOCYTOPENIA TO BAL THERAPY. L. M. Lockie, B. M. Norcross, and C. W. George. From the University of Buffalo School of Medicine, and Buffalo General Hospital. J. A. M. A. 133: 754-755, 1947.

BAL (British Anti-Lewisite; 2, 3 dimercaptopropanol) has recently been widely used in the treatment of toxic reactions caused by arsenic and mercury, with excellent results. The trial of this substance in poisoning due to other metallic substances was therefore to be expected. Apparently, the toxicity of these elements depends upon their inactivation of sulfhydryl systems, and the efficacy of BAL is believed to be due to its ability to reactivate these systems.

In the present report the authors discuss 2 patients with rheumatoid arthritis who developed hematologic abnormalities during gold therapy. The first patient showed thrombocytopenia with bleeding into the skin, gums, and brain. After unsuccessful use of transfusions, liver extract, folic acid, ascorbic acid, and vitamin K, therapy with BAL was instituted, with a prompt remission of the thrombocytopenia and subsequent complete recovery. In the second patient, granulocytopenia developed during gold therapy, and responded to treatment with BAL.

The same issue of the J. A. M. A. contains two other reports of the use of BAL to counteract various toxic gold reactions, chiefly dermatitides (pp. 749-752; 752-755). In one of these reports it was found that an increased excretion of gold was present during therapy with BAL, and the suggestion was made that BAL removes the gold which is inactivating sulfhydryl systems and allows its excretion from the body. With reactivation of these systems, toxic reactions disappear. It is of interest that the same physiologic abnormality seems to produce various types of toxic reaction, including disappearance of megakaryocytes and granulocytes in the bone marrow.

S. E.

IS PROTHROMBIN A UNITARY PRINCIPLE OR A COMPLEX? E. C. Loomis and W. H. Stegers. From the Research Laboratory, Parke Davis and Co.; and Wayne University School of Medicine, Detroit. Am. J. Physiol. 148: 563-567, 1947.

In 1945, Seegers, Loomis, and Vendenbelt isolated prothrombin and demonstrated that it was a homogeneous protein (Arch. Biochem. 6: 85-95, 1945). After detailed investigations, they were unable to find two separate prothrombin factors. The substance which they isolated was, in itself, both chemically and physiologically complete. They were at a loss, therefore, to understand the occurrence of two separable components, prothrombin A and prothrombin B, as postulated by Quick; although they were able to confirm his results when using his own methods. In an attempt to determine wherein the error in interpretation might lie, further experiments with plasmas were undertaken.

The present report, which details some of these investigations, demonstrates that "prothrombin A" is in reality fibrinogen, and that "prothrombin B" corresponds to pure prothrombin, which is a single compound and not a mixture of two complexes. There were three critical experiments:

(1) Stored beef plasma retained its prothrombin potency when measured by the two-stage test of Warner, Brinkhous, and Smith (Am. J. Physiol. 113: 667, 1940), but seemed to lose it within a week when measured by the Quick test. Apparently, then, the amount of prothrombin was constant; but its activity diminished with time.

(2) When prothrombin was quantitatively inactivated in plasma which was then allowed to stand, the prothrombin content increased to a normal value in eight days by the two-stage measurement, but remained absent by the Quick test. The addition of fresh prothrombin-free plasma did not affect the two-stage assay, but changed the Quick value to 100 per cent. Again, the interpretation seemed reasonable: that fresh plasma added something needed to activate prothrombin already present; and that the Quick determination measured, not the amount of prothrombin present, but the rate of its conversion into thrombin.

(3) The addition of fibrinogen in the latter experiment, in place of prothrombin-free fresh plasma, had an effect similar to that of the fresh plasma.

The conclusions are reached that, in Quick's assay method, fibrinogen ("prothrombin A") and not true prothrombin ("prothrombin B") was the compound denatured during refrigerator storage, and that the postulate that prothrombin is a union of two complexes, each of which, in itself, is inactive, is invalid. The authors discuss in detail the reasons for Quick's arrival at this conclusion, and comment on

deductions since published on the basis of the concept that prothrombin is a complex substance. Their demonstration of the unitary nature of prothrombin is completely convincing.

S. E.

THE IMPORTANCE OF BLOOD CHANGES IN CORONARY OCCLUSION. *W. M. Cameron, J. H. B. Hilton, S. R. Townsend, and E. S. Mills.* From the Department of Medicine, the Montreal General Hospital, Montreal, Quebec. *Canad. M. A. J.* 56: 263-267, 1947.

In 15 patients suffering from coronary occlusion the authors found no constant changes in blood volume, circulation time, coagulation time, prothrombin concentration, or coagulability of the blood as measured by a modified Waugh-Ruddick test. Plasma proteins were within normal limits in all cases, but 6 of 15 patients showed significant hemoconcentration on admission.

It is unfortunate that the series reported is too small to justify definite conclusions.

L. E. Y.

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# BOOK REVIEWS

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*Le Malattie del Sangue*. By A. FERRATA AND E. STORTI. Milan: Societa Editrice Libraria, 1946. Pp. 732.

A much needed, authoritative, up-to-date text book in Italian, *Le Malattie del Sangue* by A. Ferrata and E. Storti\* is presented by the same Societa Editrice Libraria which in 1912 published Ferrata's well known *Normal and Pathological Morphology of the Blood* and later several editions of *Hemopathy*, considered one of the most complete, original, and well documented works on hematology.

The senior author, A. Ferrata, recently deceased, who stands out as one of the foremost hematologists of our day, needs no introduction to prospective readers of the new book, a companion volume to his larger work, *Hemopathy*.

Only two devoted friends, the publisher and Dr. Storti, the co-author, could combine to make a volume so representative of the work of this scholar. In the words of G. DiGuglielmo, who wrote the preface to the book, "Blood diseases can be considered the spiritual legacy of A. Ferrata." The forty years of his clinical experience are concentrated in the 750 pages of this complete, clearly written, and beautifully illustrated book.

The thirteen chapters easily carry the reader from morphology, genesis, and physiology of the blood and of the blood-forming organs to the most modern technics for the hematologic investigation of the anemias, the myeloses, the hemorrhagic diatheses, etc. Each chapter is so replete with significant observations that it is difficult to single out subjects for review.

Many controversial points, such as that of embryonal hematopoiesis, in its three stages, mesoblastic (prehepatic), hepatic, and medullary, are treated with skill and good judgment. Dr. Storti's original work has contributed a good deal to the success of this difficult chapter in which the hemoglobin-containing cells of the first generation (megaloblastic cells) are clearly shown in excellent illustrations.

The different types of anemias are clearly presented, with inclusion of the most recently studied forms. A good description is given of the so-called "achrestic" anemia (Wilkinson and Isaacs), the cause of which is attributed to impeded utilization or mobilization of the antianemic factor. In the large group of the hypochromic anemias, special emphasis is given to the "achylic chloro-anemia" of Kazzelson, Reimann, and Weiner, which is dealt with as a separate entity among the other forms of hypochromic anemias of unknown cause.

Among the congenital splenomegalic hemolytic anemias, the type described by Greppi and Micheli is singled out from the classical Minkowski-Chauffard variety mainly on the basis of the increased osmotic resistance of the erythrocytes and the lack of response to splenectomy.\* "Lederer-Brill" acute hemolytic anemia is also given a separate place in the general classification, although in the opinion of the reviewer this condition might have been better identified with the acquired hemolytic icterus of the older writers of the French and Italian schools.

In the chapter, "Myeloses," prominence is given to erythroleukoses and to the acute and chronic erythremic myeloses, characterized by a systemic anaplastic proliferation of normo-erythroblastic cells. In the acute forms, ending fatally in one to four months, proerythroblasts and erythroblasts prevail, whereas in more protracted chronic forms, less immature cells of the erythrocytic series, orthochromatic and polychromatophilic erythrocytes are predominant. The Italian school of hematologists has contributed much to this concept, which is almost ignored in the English-American literature. "Cooley's anemia" is unfortunately considered a familial variety of chronic erythremic myelosis!

Aplastic anemias, from the symptomatic standpoint, are classified as pancytopenic, granulocytopenic, anemic, and thrombocytopenic, in agreement with the classification generally followed by the Americans

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\* These authors failed to note that this type is a mild form of the Mediterranean anemia known as Cooley's anemia.

schools. Atrophy of bone marrow, arrest in cellular maturation, and impeded entrance into the blood stream of the newly formed hematic cells are considered among the factors underlying the condition.

The work as a whole is excellent and is highly recommended both to the specialist and as a reference book to the general practitioner. A selective, impartial bibliography is appended to each chapter, including the most important contributions of the American writers.

An English translation of this thoroughly fascinating and scholarly volume might well be in order.

*Rh: Its Relation to Congenital Hemolytic Disease and to Intragroup Transfusion Reactions.* By EDITH L. POTTER, M.D., Ph.D. The Year Book Publishers, Inc., Chicago. 1st Ed. Pp. 344.

Few subjects in recent years have aroused the widespread interest that has followed the discovery of the Rh-Hr complex and its application to clinical medicine. This interest has led to a profusion of articles on various phases of this subject and to some confusion as to nomenclature, methods of testing, and therapy. It was inevitable that a book on this topic would appear, and it is fortunate that so excellent a review has been written by an author with both wide pathologic and authoritative clinical experience in this field.

Dr. Potter's book is not only timely and complete but also carefully and critically written. The subject matter is logically presented. A short history of the discovery of the Rh factor is followed by a lucid discussion of Rh-Hr antigens and antibodies. The main emphasis is placed on the role played by Rh incompatibility in intragroup hemolytic transfusion reactions and in hemolytic disease of the newborn, together with excellent descriptions of the clinical and pathological findings in these conditions, their prevention, and therapy. The final chapter deals with methods of testing for the Rh factor and anti-Rh antibodies. Outstanding is the clear exposition of the theories of the genetic inheritance of the Rh-Hr complex, the description of the pathologic findings, the excellent photographic illustrations, and the complete bibliography of nearly 800 titles, which includes almost every significant article on this subject in the world literature up to 1946.

In short, this book is not only opportune but so clear, concise, and rationally organized that it can be strongly recommended to anyone—student, practitioner, or specialist—seeking a logical exposition of an involved and expanding subject.

*A Color Atlas of Hematology.* By ROY R. KRACKE, M.D. J. P. Lippincott Co., Philadelphia. Pp. 204, 32 plates in color.

Dr. Kracke states in his preface, "The preparation of this volume has been prompted by the belief that there is a widespread need for a color atlas of hematology, particularly among medical students, laboratory workers and general practitioners of medicine. The various works hitherto available in this field have either originated in foreign countries or have been incomplete because of coverage of only certain phases of the subject." There can be no doubt as to the validity of these statements.

All the color plates in the *Atlas* have been taken from Dr. Kracke's larger work, originally published with Hortense Garver, *Diseases of the Blood and Atlas of Hematology*. That work contained 54 color plates and the present reviewer stated in 1942\* that "the pictures of blood smears and cells far outrank anything else in this country and for the *Atlas* alone, the book is worth owning. The lithography is unusually good."

Unfortunately, one cannot say the same for the present condensed *Atlas*, in which the lithography is often very poor. Comparison of identical plates from the large work published in 1941 with those in the present *Atlas* indicates that either the lithographic plates have deteriorated in the past six years or else (what is more likely) that present day postwar lithography leaves much to be desired. This is particularly true of the red blood cells, which often appear muddy, with poor color application (cf. particularly plates 16, 17, 18). Plates 28, dealing with acute myeloid leukemia, and 29, with infectious mononucleosis, have come out particularly badly and are quite in contrast with such rather good "bright" plates as 27, dealing with acute myeloid leukemia, and 23, with chronic myeloid leukemia.

\* N. E. J. Med. 227: 324, 1942.

It must also be stated that the 10 preliminary plates concerned with the origin and development of the blood cells fare badly. One must, as ever, disagree with Dr. Kracke both as to his depiction of the "megaloblast" and as to his use of the word as a designation of the precursor of all the red blood cells. His definition reads, "A large nucleated red cell with a cart wheel and a reticulate nucleus when stained. A nucleated precursor of the normoblast."

Excellent legends and short descriptions accompany the various plates. Sections on "Definitions of Hematologic Terms," "Hematologic Technic" and "Summary of Hematologic Findings" are presented. The reviewer confesses a preference for Dr. Kracke's earlier and much more important volume.

# BLOOD

## *The Journal of Hematology*

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### HEMOPHILIA

#### A REPORT OF THE MECHANISM OF THE DEVELOPMENT AND ACTION OF AN ANTICOAGULANT IN TWO CASES\*

By CHARLES G. CRADDOCK, JR., M.D., AND JOHN S. LAWRENCE, M.D.

THE purpose of this report is to present two cases of hemophilia in whom an anticoagulant was found. A study of the mode of development and action of the anticoagulant and a hypothesis as to its clinical significance are presented.

There are two reports in the medical literature which describe the development of a similar anticoagulant in hemophilia. The first of these was by Lawrence and Johnson,<sup>1</sup> the patient in their report being one of the two (W. P.) included in this paper. A similar patient was reported in 1943 by Munro and Jones<sup>2</sup> and these authors noted that the anticoagulant developed after repeated transfusions, at which time further transfusion caused neither a reduction in clotting time nor clinical improvement. They suggested that "the numerous transfusions were responsible for the development of this anticoagulant."

The anticoagulant in the case of Lawrence and Johnson was found to be heat stable and nondialyzable through collodion. Relationship to heparin was ruled out since anticoagulant activity was not inhibited by protamine.<sup>3</sup> Munro later published detailed studies on the nature of the anticoagulant in his case.<sup>4</sup> It had the same general physical properties as that in the patient of Lawrence and Johnson. He carried the analysis further by plasma fractionation and showed clearly that the anticoagulant activity was associated with the globulin fraction of the plasma. In another report<sup>5</sup> on this same case he repeated his fractionation using the electrophoretic technic and showed that the anticoagulant activity was associated with the gamma globulin fraction.

#### REPORT OF CASES

*Case 1* (No. 27899). W. P., a fifty year old unmarried male, is the same case previously reported by Lawrence and Johnson.<sup>1</sup> He is a typical hemophiliac, conforming to the usual clinical picture and showing the abnormalities in the clotting mechanism diagnostic of hemophilia. The family history is positive, there being one maternal uncle and one brother with the disease. Since the last report he has had recurrent episodes of bleeding into the joints, from the genito-urinary tract and from the gastrointestinal tract.

From the Department of Medicine of the University of Rochester School of Medicine and Dentistry and the Medical Clinic of the Strong Memorial and Rochester Municipal Hospitals, Rochester, New York.

\* This document is based on work performed under Contract No. W-7401-eng-49 for the Atomic Energy Project at the University of Rochester.

He has also developed hypertension and hypertensive heart disease with occasional bouts of paroxysmal auricular fibrillation. During the periods when bleeding occurred, little benefit was derived from transfusion with fresh blood or plasma, and joint bleeding was treated symptomatically and by rest, with spontaneous remission of symptoms. In November 1945 a test for the presence of a circulating anticoagulant was negative.

From December 1945 until March 1946 the patient was in the hospital for 3 months because of severe continuous hemorrhage per rectum. During this time he received in all 30 transfusions of whole fresh blood, each consisting of 500 cc. In spite of almost daily transfusion at one point, he did not improve and no effect on the coagulation time was recorded. The latter consistently varied from 60 to 120 minutes. Unfortunately during this time no tests for a circulating anticoagulant were performed. Finally, the patient began to improve slowly and once the bleeding ceased, the red cell count and hemoglobin returned rapidly to normal. Transfusions had been discontinued during the period of improvement. No abnormality in the gastrointestinal tract was detected at that time.

The last transfusion of whole blood was given on February 9, 1946. After this he was seen repeatedly in the clinic, usually for anginal pain associated with his heart disease. He received no more injections of blood or plasma until the present admission.

He was tested on September 9, 1946 for the presence of a circulating anticoagulant. The test was negative (table 1).

On April 15, 1947 he was readmitted to the hospital because of bleeding per rectum of 3 to 4 hours duration. At this time his red cell count and hemoglobin were low (2.96 million per cubic millimeter and 9.0 grams per cent respectively). He was pale, dyspneic, and suffered repeated attacks of precordial pain. He was immediately started on multiple transfusions of whole fresh blood. This was done before a test for circulating anticoagulant was obtained. In spite of 6 transfusions with 500 cc. fresh whole blood each, no improvement by either clinical or laboratory findings occurred although no evidence of intravascular hemolysis appeared. The ischemic pain became progressively worse, the dyspnea increased and the red blood cell count and hemoglobin fell steadily to a level of 1.3 million per cubic millimeter and 4.5 grams per cent respectively. At this point the anticoagulant effect of his blood on normal blood was very marked. Clotting times were performed repeatedly at one-half hour intervals after transfusion and no effect was noted, the clotting time remaining around 60 to 70 minutes.

Because of findings to be discussed later, it was felt advisable to stop transfusions with whole blood and instead to give only washed red cells. After receiving 500 cc. of red cells he showed some signs of symptomatic improvement. Another transfusion of whole blood given inadvertently caused an immediate recurrence of symptoms. After this he received 500 cc. units of washed red cells and showed steady symptomatic improvement. However, he continued to bleed, the clotting time remained prolonged, and the circulating anticoagulant persisted.

Gradually he improved, the red blood cell count and the hemoglobin rising and the attacks of anginal pain disappearing. At the time of this report he is completely asymptomatic and bleeding has ceased.

*Case 2* (No. 143378). D. M., a twenty-one year old white male, is a known hemophiliac since the age of 1 year. He has a completely negative family history. Bleeding as a boy was usually from a traumatized tongue or into joints. At the age of 19 he developed hematuria for which he received 9 plasma transfusions without benefit. The coagulation time remained unchanged. Consequently he was admitted to Strong Memorial Hospital for study, 9 months later, on November 15, 1944. Laboratory findings, including Quick's differential plasma centrifugation clotting time, were typical of hemophilia at that time.

A test for the presence of a circulating anticoagulant performed before any injections of blood or plasma was negative. Response to injections was only slight, the clotting time dropping from about 1 hour to 30 minutes and returning to its original level in from 4 to 6 hours. He received in all, 2 injections of fresh plasma and one direct transfusion of whole blood. The test for a circulating anticoagulant was not repeated after these injections.

Between November 1944 and the present admission he suffered only occasional joint hemorrhages and was in good general health until December 1946. At that time he began to have intermittent gross urinary bleeding. Shortly thereafter he had a mild pharyngitis followed by gross hematuria. He finally received a transfusion of whole blood 11½ weeks before admission, but the bleeding became more profuse. He was taken to Wilson Memorial Hospital, Binghamton, New York where he received 17 direct trans-

fusions and several units of antihemophilic globulin. Bleeding finally slowed and he was sent home, where, although bleeding continued, no clots were passed. Because of the continued bleeding he was admitted to Strong Memorial Hospital on April 15, 1947 for study.

Physical examination at that time was essentially negative except for partial ankylosis and contractures of both knees and left elbow. Laboratory studies showed a hemoglobin of 5.5 grams per cent, red cell count 2.7 million per cubic millimeter, white cell count 7,000 per cubic millimeter with a normal differential. The urine was grossly bloody with a few threadlike clots. Coagulation studies were normal except for a clotting time of 68 minutes. The Quick differential centrifugation plasma clotting test was positive for hemophilia.

Because of the history of refractoriness to transfusion with whole blood it was felt that a trial injection of whole blood should be given to see if such a refractory state actually existed. If he responded it could be assumed that he was the usual type of hemophilic, but if no response resulted then it could be assumed he probably had some abnormal element in his blood which mitigated the beneficial effect of whole blood. Consequently, on April 16, 1947 he was given 200 cc. fresh whole blood. The clotting time dropped from an average of 68 minutes to 12 to 14 minutes in a half hour. However, after 17 hours, when the next test was performed, the clotting time was again 68 minutes. It was felt he probably did not have an anticoagulant but merely received only transient benefit from fresh blood. Therefore it was decided to treat his anemia and bleeding vigorously.

He was given transfusions on 2 successive days and the coagulation times checked at a half hour, 1 hour, and thereafter at 1 hour intervals until the clotting time returned to normal. It was found that the effect on the clotting time was evanescent, not lasting more than one half hour after completion of the transfusion. He was then given one vial of antihemophilic globulin (Fraction I of Cohn, Squibb) and the clotting times checked every 15 minutes for 2½ hours. A reduction from 80 to 95 minutes to 30 minutes occurred in a half hour, but there was a quick rise to the previous level. Two days later he was given 2 vials of antihemophilic globulin and the clotting times again followed. This time no effect whatsoever on the clotting times was recorded. It was also noted that the clotting times had risen to about 120 to 135 minutes, whereas before they had averaged around 68 minutes. The urine continued to be grossly bloody.

It became apparent at this time that an alteration in the patient's coagulation mechanism had probably developed as a result of the transfusions and injections of antihemophilic globulin. Therefore on April 22, 1947 a test for a circulating anticoagulant was performed. This was readily demonstrated (table 1). Tests were repeated on the following day with the same result. Consequently studies of this anticoagulant were carried out, as will be described. Therapeutically, the patient was given 3 transfusions of washed red cells over the next 2 week period. Studies of renal function (PSP, NPN) were normal and an intravenous pyelogram showed no definite genito-urinary pathology, but was not clear because of blood in the pelvis of the left kidney.

The patient quickly improved symptomatically as the anemia was corrected. However, he continued to bleed from the left kidney, though not as severely. He was finally discharged to Dr. R. J. McMahon to be followed and treated symptomatically.

The *technic* of performing the tests for the circulating anticoagulant in these cases was as follows.

1. Clean graduated 15 cc. centrifuge tubes were rinsed with saline before using.
2. Venipuncture was performed simultaneously on the patient and a normal subject. 5 cc. of sterile saline in each syringe was injected simultaneously into each, and 10 cc. of blood withdrawn without stasis at exactly the same time and rate from the patient and the normal subject.
3. The two specimens of blood were then put into the tubes as follows:

Tube .....	1	2	3	4	5	6	7	8	9	10
Patient's Blood (cc.).....	0.0	0.0	0.0	0.4	0.8	1.2	1.6	2.0	2.0	2.0
Normal Blood (cc.).....	2.0	2.0	2.0	1.6	1.2	0.8	0.4	0.0	0.0	0.0

The clotting times were then recorded, the test being run in a water bath at  $37.5^{\circ}\text{C}$ . and all tubes tilted in a rack at 1 minute intervals.

Although this is a crude technic it was found that demonstration of the anticoagulant was easier using whole blood in this manner than by using plasma or serum. This was especially true in the case of D. M. where the anticoagulant activity was relatively weak.

Results of this test using the blood of the 2 patients are shown in table 1. It will readily be seen that definite prolongation of the clotting time of normal blood was caused by mixing with the blood of either of these patients.

The technic employed is the same as that used by Lawrence and Johnson in their original studies on patient W. P. At that time they repeated this test on 3 ordinary hemophiliacs as a check, and in no case was the clotting time of normal blood prolonged in any of the tubes. We repeated the test on 2 hemophiliacs who had not been transfused for over a year with negative results.

The anticoagulant was also shown using plasma in a manner similar to the protocol of Munro.

TABLE 1.—*Demonstration of Anticoagulant Effect of Blood of Patients W. P. and D. M. on Normal Blood*

Tube Number		1	2	3	4	5	6	7	8	9	10
Patient's Blood (cc.)	.....	0.0	0.0	0.0	0.4	0.8	1.2	1.6	2.0	2.0	2.0
Normal Blood (cc.)	.....	2.0	2.0	2.0	1.6	1.2	0.8	0.4	0.0	0.0	0.0
Patient	Date	Clotting Time (min.)									
W. P.*	9/ 9/46	8	8	8	7	8	8	8	70	71	65
W. P.	4/24/47	6	6	7	8	10	16	36	60	52	60
W. P.	4/29/47	7	7	6	10	12	18	40	58	56	55
D. M.	4/22/47	8	6	6	14	15	16	17	75	73	75
D. M.	4/23/47	6	6	6	9	14	15	16	100	95	95

\* This test was performed eight months after the last transfusion.

#### PROCEDURE

The blood was collected by venipuncture and mixed with one ninth its volume of 0.1 M sodium oxalate. Centrifugation for 1 hour at 2000 r.p.m. was carried out. Various amounts of the plasma were mixed with normal plasma prepared in the same manner (table 2). Recalcification was accomplished by adding 0.025 M calcium chloride in amounts equal to the amount of plasma present, the total volume being adjusted to 1.2 cc. by the addition of the requisite amount of 0.15 M sodium chloride.

It can be seen from these data that the anticoagulant effect was much stronger in the case of W. P. than in the case of D. M. However, the plasmas of both show a definite inhibitory effect on coagulation when the concentration of their plasma reaches 27 to 35 per cent of the total plasma.

It was realized from the clinical course of these patients that an alteration of the coagulation mechanism of each was being brought about by transfusions of whole blood and/or injection of antihemophilic globulin. From the data given, it would

seem this was due to the development of a circulating anticoagulant, as described by Munro.<sup>3</sup> It was felt that further analysis of the nature of the anticoagulant and the manner in which it acted was indicated.

Steps were then taken to determine which phase of clotting was inhibited by this anticoagulant. These will be described briefly.

The prothrombin concentration of each patient's plasma was determined by the serial dilution modification of Quick's test. The prothrombin concentration of W. P. was 80 per cent of normal and that of D. M. 100 per cent of normal. These concentrations are of course high enough to exclude prothrombin deficiency as a cause of the bleeding, and since conversion to thrombin occurred in the normal time, antiprothrombin also could be excluded.

Tests were made for the presence of an antithromboplastin. According to Tocantins, hemophilic blood contains an excessive amount of antithromboplastin.<sup>6</sup> However, in order to demonstrate it, special technics must be used or it will be destroyed. No special technic was used in handling this blood and yet the anticoagulant effect remained. This would therefore seem to be a substance different from the anti-

TABLE 2.—*The Anticoagulant Action of the Patient's Plasma on Normal Plasma*

Patient's Plasma	Normal Plasma	Sodium Chloride 0.15 M	Calcium Chloride 0.025 M	Coagulation Time	
				W. P.	D. M.
cc.	cc.	cc.	cc.	min.	min.
0.00	0.40	0.40	0.40	3	2
0.05	0.40	0.30	0.45	3	2
0.10	0.40	0.20	0.50	4	3
0.15	0.40	0.10	0.55	15	4
0.20	0.40	0.00	0.60	38	16
0.20	0.20	0.40	0.40	50	18
0.40	0.10	0.20	0.50	55	20
0.40	0.00	0.40	0.40	75	30

thromboplastin described by Tocantins. It was felt that if the anticoagulant effect was still due to an antithromboplastin after handling the blood in the routine manner, it would be detectable by less sensitive means. Consequently a thromboplastin solution of known potency was serially diluted and the prothrombin times established, using each dilution with normal plasma. In this way progressively longer prothrombin times were found with increasing dilutions of thromboplastin. If antithromboplastin were present in greater concentrations in the patient's blood than in normal blood, a significant differential in the prothrombin times of the patient's and normal plasma so tested should be readily demonstrated. This was not found to be the case.

From these data it can be assumed that antithromboplastin was not present in enough strength to explain the anticoagulant activity of the patient's blood.

Determination of antithrombin activity of the plasma was carried out, although its presence would be doubtful in view of a normal rate of conversion of prothrombin to thrombin. A standard thrombin solution (Thrombin-Upjohn) was so diluted that 1 cc. contained 2 units of thrombin (the potency of the thrombin was such that 2 units clotted 1.0 cc. of oxalated human plasma in  $15 \pm 0.5$  seconds at  $28^\circ \pm$

2° C.). One cc. of the thrombin solution was then added to 1 cc. samples of normal and both patients' plasma. In each case clotting repeatedly occurred in 15 to 16 seconds. Antithrombin activity was therefore absent from the plasma of each of these patients.

The presence of antifibrinogen was felt not to be probable since the final clot which formed was completely normal as far as quantity, strength, and elasticity were concerned. Specific tests for the presence of an antifibrinogen were deemed unnecessary.

TABLE 3.—*Test for the Presence of Antithromboplastic Activity in Plasma of Patients*

Thromboplastin Solution	Clotting Time		
	Normal	W. P.	D. M.
<i>dilution</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>
Undil.	15	17	14
1/2	15	17	14
1/4	16	17	15
1/8	21-23	22	10-21
1/16	22-25	25	22
1/32	25-28	29	23
1/64	28-30	30	25
1/128	30-32	33	28
1/256	30-35	35-38	30-31

In each case 0.1 cc. thromboplastin solution was added to 0.1 cc. plasma. Recalcified with 0.05 M Calcium Chloride. Performed at 37.5° C.

TABLE 4.—*Effect of Toluidine Blue on the Activity of the Anticoagulant in the Plasma of the Patients*

Amount of Toluidine Blue in mg. per 0.2 cc. of saline	Amount of Plasma	Calcium Chloride 0.025 M	Clotting Time		
			Normal	W. P.	D. M.
	<i>cc.</i>	<i>cc.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
0.0000	0.2	0.2	2.5	14.0	11.0
0.0036	0.2	0.2	2.0	14.0	11.0
0.0062	0.2	0.2	2.25	14.0	11.0
0.0125	0.2	0.2	2.25	14.0	11.0
0.0250	0.1	0.2	3.0	15.0	12.0
0.0500	0.2	0.2	4.0	17.0	13.0
0.1000	0.2	0.2	8.0	18.0	16.0
0.2000	0.2	0.2	9.0	18.0	17.0

The presence of an anticoagulant of the heparin type was sought by *in vitro* studies with toluidine blue. This dye, which in itself is an anticoagulant in higher concentrations, exerts an inhibitory effect on heparin if present in the correct concentrations, as shown by numerous workers. The optimal amount of dye necessary to inhibit heparin, as shown by Jacobson and Allen,<sup>14</sup> is about 6 gamma of dye per 10 gamma of heparin. The procedure used was as follows:

A solution of 100 cc. saline containing 0.1 gram of dye (the dye being 64 per cent toluidine blue by dry weight) was used. Serial dilutions were made and 0.2 cc. of

each dilution of the dye solution was added to 0.2 cc. of plasma and the clotting times recorded after recalcification.

It will be seen from table 4 that when the concentration became higher than 0.0125 mg. the anticoagulant effect of the dye on normal plasma became evident. It is also apparent that the toluidine blue in concentrations which would have inhibited heparin, if it were present, had no shortening effect on the clotting time of the plasma of either patient. It can therefore be concluded that the activity of the anticoagulant is probably not due to the presence of heparin. This was of particular interest in view of the recent finding that substantial amounts of heparin are found in the blood of animals after large amounts of irradiation. The anticoagulant in this case would seem to be of an entirely different nature than that found after x-ray.

TABLE 5.—*The Relative Anticoagulant Activity of Fractions of Patient's (W. P.) Plasma Separated Electrophoretically*

Fraction of Plasma		Normal Plasma	Isotonic Saline	Calcium Chloride 0.025 M	Clotting Time
no.	cc.	cc.	cc.	cc.	min.
I	0.1	0.2	0.2	0.2	8
I	0.2	0.1	0.3	0.1	10.5
II	0.1	0.2	0.2	0.2	7
II	0.2	0.1	0.3	0.1	10
III	0.1	0.2	0.2	0.2	5.5
III	0.2	0.1	0.3	0.1	6.5
IV	0.1	0.2	0.2	0.2	2.5
IV	0.2	0.1	0.3	0.1	2.5
IV	0.3	0.1	0.2	0.1	2.5
IV	0.4	0.1	0.1	0.1	2.5
		0.2	0.2	0.2	2.5
		0.2	0.2	0.2	2.5

Electrophoretic fractionation of the plasma of one of the patients (W. P.) was performed by Dr. Eric Alling as follows: The fractionation was carried out in the tall form of the 11 ml. Tiselius cell, in 0.1 molar veronal buffer of pH 8.5. After maximal separation of the peaks a blunt needle was slowly lowered into the cell to successively increasing depths controlled by visual observation through the optical system. Each fraction was drawn off very slowly by means of a syringe operated by the Klett motor-driven compensator.

From the descending limb of the cell the following fractions were withdrawn:

Fraction I: Gamma globulin.

Fraction II: Gamma globulin plus fibrinogen plus beta globulin.

Fraction III: Gamma globulin plus fibrinogen plus beta plus alpha<sub>2</sub> plus alpha<sub>1</sub> globulin plus albumin.

From the ascending limb only one fraction was withdrawn. This fraction contained all components except gamma globulin and was designated as Fraction IV. Each of these fractions was then tested for its anticoagulant activity on normal plasma as shown in table 5.

The anticoagulant activity was present in all the fractions which contained gamma globulin, but was absent from Fraction IV which lacked only gamma globulin. Therefore the anticoagulant was associated with the gamma globulin fraction of plasma.

It was apparent that the anticoagulant had several characteristics which would seem to indicate that its appearance and action were on an immunologic basis. In the first place, the anticoagulant became evident after repeated injections of whole blood or antihemophilic globulin. Also the anticoagulant proved to be a gamma globulin, as previously shown by Munro.

Workers at the Thorndike Memorial Laboratory<sup>7-9, 11-13</sup> have shown that normal plasma contains a factor which is deficient in the plasma of hemophiliacs. They have found this substance to be associated with the globulin fraction of the plasma protein. This globulin fraction is contained in Fractions I and III-2 (II of Cohn). When Fraction I is given intravenously to hemophilic patients, it causes a marked reduction in clotting time, similar to that produced by whole blood. This globulin fraction has been called antihemophilic globulin and is necessary for coagulation of blood to proceed at the normal rate. The precise function of this globulin and the point at which it takes part in the coagulation mechanism have not yet been elucidated. However, it has been suggested by Quick and others that the fundamental alteration in the coagulation of hemophilic blood is the failure of the platelets to agglutinate and break down to release thromboplastin. Since the only abnormality yet detected in hemophilic blood is a deficiency of the globulin substance called antihemophilic globulin, it is possible that the two abnormalities are related and that antihemophilic globulin must be present for the breakdown of platelets to occur. A deficiency of this globulin, as in hemophilia, may account for the "greater stability" of the platelets which do not readily agglutinate, break down, and release thromboplastin for the initiation of clotting.

Since this protein factor is deficient in hemophilic blood, it was thought conceivable that this lack offered the basis for the development of the anticoagulant found in the blood of these patients. In other words, because these patients were lacking in a globulin factor normally present in blood, repeated injections of this globulin either in whole blood or plasma, or in the more concentrated form of Fraction I of Cohn might elicit an antibody response against this protein which would eventually be manifest as an anticoagulant.

If the development of antibody against antihemophilic globulin did occur, demonstration of it should be possible. Precipitin tests were therefore set up using the serum of each patient and Fraction I of Cohn (Cutter). Amounts of Fraction I containing 0.2 grams protein were used, the relative amounts of fibrinogen and antihemophilic globulin contained not being known. This dried material was dissolved in 5 cc. of sterile distilled water, thus making a 4 per cent protein solution. The pH was adjusted to 7.0 to 7.5. Precipitin tests were performed at room temperature and read at one-half hour and one hour intervals. Dilutions of antigen ranging from 1:1 to 1:1280 were used, the dilution being doubled in each tube.

It will be seen that the titres obtained with both patients' sera against Fraction I were significantly higher than those obtained with normal or ordinary hemophilic serum. The titres obtained in the patients' sera tested against normal plasma

are low but suggest that the sera contained antibodies against some element in normal plasma also. Titrations against each other's plasma as controls were negative.

Similar control precipitin tests were set up using the sera against:

1. A solution of purified fibrinogen (Parke-Davis)
2. A solution of purified gamma globulin (Parke-Davis)

Both of these antigens gave negative results.

The serum of a non-hemophilic patient who had received multiple transfusions was tested in the same manner with failure to demonstrate any precipitins. These controls indicate that the precipitins found only in the sera of the two patients were specific for antihemophilic globulin.

TABLE 6.—*Showing Presence of Precipitins in Sera of the 2 Patients against Antihemophilic Globulin (Fraction I of Cohn)*

Serum	Antigen				
	Antihemophilic Globulin (Fraction I)	Normal Plasma	D. M.'s Plasma	W. P.'s Plasma	Ordinary Hemophilic Plasma
Normal.....	1/1	0	1/10	1/10	0
D. M.....	1/160	1/40	0	0	1/10
W. P.....	1/320	1/40	0	0	0
Ordinary hemophilic	0	0	0	0	0

TABLE 7.—*The Anticoagulant Effect of Serum of Each Patient on Normal Blood*

Normal Blood	Patient's Serum	Clotting Time	
		When W. P.'s Serum added	When D. M.'s Serum added
cc.	cc.	min.	min.
0.4	1.6	15	11
0.8	1.2	13	9
1.2	0.8	10	6
1.6	0.4	8	5
2.0	0.0	5	5
2.0	0.0	5	5

These tests gave repeatedly similar results. The precipitin titres remained at 1/320 for W. P. and at 1/160 for D. M. The latter patient, because of his better condition, was given 3 injections of antihemophilic globulin (one vial each) on 3 successive days after the precipitin test was performed. Despite this, the titre did not change over the following 2 week period, nor did the anticoagulant activity become more intense.

Since the antibody was present in the serum, as shown by the precipitin tests, it should have been possible to demonstrate anticoagulant activity of the serum. Although some effect was noticed when the serum was mixed with normal plasma, the change was most strikingly brought about by mixing with whole blood without decalcifying.

As shown in table 7, serum from these patients did not have as strong an antico-

agulant effect as did either whole blood or plasma. The most striking alteration noted, however, was that the clotting process once it was begun was markedly slowed. That is, the length of time elapsed between initiation of clotting and completion of the process was much greater than for normal blood alone. A few strands of fibrin would form and then gradually the clot would become larger and firmer; the phenomenon resembling closely that seen when hemophilic blood clots.

That the anticoagulant which developed in the circulating blood of these patients exerted its action by directly inhibiting antihemophilic globulin was also shown by the following tests.

Taylor, Davidson, Tagnon, et al.<sup>10</sup> have demonstrated that antihemophilic globulin has the ability to lower the coagulation time of hemophilic blood or plasma if added to it in vitro. This was repeated in the case of an ordinary hemophilic by us and the effect was found to be quite striking. However the globulin had no acceleratory effect on the clotting time of the patients' blood (table 8). An experiment was then set up in which the antihemophilic globulin, as contained

TABLE 8.—Effect of Antihemophilic Globulin on the Coagulation Time in Vitro of the Blood of an Ordinary Hemophilic and one of the Patients (W. P.)

Amount of Blood	Isotonic Saline	Antihemophilic Globulin (Fraction I of Cohn)				Clotting Time	
		Undil.	1/10	1/100	1/1000	Ordinary Hemophilic	Patient W. P.
cc.	cc.	cc.	cc.	cc.	cc.	min.	min.
2.0	0.2					40	62
2.0	0.2					36	62
2.0	0.2					40	63
2.0	0.1	0.1				13	82
2.0	0.1		0.1			7	64
2.0	0.1			0.1		7	62
2.0	0.1				0.1	11	58

in Fraction I of Cohn, was first incubated with a small amount of serum from each of these patients. When this was done, the clot-accelerating activity of the antihemophilic globulin on hemophilic blood was lost.

#### PROCEDURE

A 4 per cent solution of Fraction I in isotonic saline was prepared. The pH of the solution was adjusted to 7.0 to 7.5 using a glass electrode pH meter. The total nitrogen of the final centrifuged solution was determined by the Kjeldahl method and found to be 2.8 grams per cent.

Serial dilutions of this solution were then made, undiluted, 1/10, 1/100, 1/1000. 0.1 cc. of samples of each dilution of the antihemophilic globulin were then added to 0.1 cc. samples of serum of the following types.

1. Normal serum
2. W. P.'s serum
3. D. M.'s serum
4. Serum from ordinary untreated hemophilic.

These mixtures of 0.1 cc. of antihemophilic globulin and 0.1 cc. of the various sera were incubated at 37.5° C. for one-half hour.

Blood was then drawn from an ordinary hemophiliac patient who had not received any transfusions or injections for over 1 year and who was known to respond to treatment in the usual manner.

This blood was then added to the tubes as shown in tables 8 and 9.

It is shown by these protocols that antihemophilic globulin alone had a marked accelerating effect on the coagulation of the blood of an ordinary hemophilic patient. Also when antihemophilic globulin was first incubated with either normal

TABLE 9.—*Effect of Antihemophilic Globulin on the Coagulation Time of Ordinary Hemophilic Blood after Incubation with Sera*

Blood of Ordinary Hemophiliac	Antihemophilic Globulin Incubated with Normal Serum				Antihemophilic Globulin Incubated with W. P.'s Serum				Antihemophilic Globulin Incubated with D. M.'s Serum				Antihemophilic Globulin Incubated with Ordinary hemophilic serum				Clotting Time
	Undil.	1/10	1/100	1/1000	Undil.	1/10	1/100	1/1000	Undil.	1/10	1/100	1/1000	Undil.	1/10	1/100	1/1000	
cc.																	min.
2.0	0.2																6
2.0		0.2															5
2.0			0.2														5
2.0				0.2													6
2.0					0.2												31
2.0						0.2											35
2.0							0.2										36
2.0								0.2									40
2.0									0.2								15
2.0										0.2							18
2.0											0.2						22
2.0												0.2					35
2.0													0.2				7
2.0														0.2			5
2.0															0.2		6
2.0																0.2	6

serum or serum from an ordinary hemophiliac this acceleratory effect on hemophilic blood was not impaired. When, however, the antihemophilic globulin was first incubated with the serum of either of the two patients who possessed the circulating anticoagulant then the ability of the globulin to accelerate the clotting time of ordinary hemophilic blood was lost.

This shows quite clearly that the action of the anticoagulant in these two cases was specifically against the antihemophilic globulin itself. Since it was previously shown that the anticoagulant was not inhibitory to any of the other components of clotting, it would seem that inhibition of this globulin factor necessary for normal coagulation is the basis of the anticoagulant activity of the blood of these patients.

## DISCUSSION

Each of these two patients exhibited a circulating anticoagulant in his blood. This anticoagulant was apparently not in sufficient concentration to be easily demonstrable until several transfusions or injections of Fraction I of Cohn, containing the substance called antihemophilic globulin, were given. This latter factor is present in normal blood or plasma and is essential for normal coagulation. Although how it is utilized or just where it enters into the clotting mechanism is unknown, it probably is important in the agglutination and lysis of platelets with liberation of thromboplastin. This globulin factor is deficient in the blood of hemophiliacs<sup>7,9-12</sup> and may account for the failure of the platelets in hemophilic blood to break down as in normal blood.

The anticoagulant in these patients could not be shown to be antagonistic to any of the components included in the classical concept of clotting. The anticoagulant was shown to be associated with the gamma globulin fraction of plasma, and in this respect was similar to the anticoagulant reported by Munro in his patient.<sup>4</sup> Evidence is presented to show that the anticoagulant which developed in these patients exerts its activity directly against antihemophilic globulin and in this way inhibits coagulation of normal blood. In view of the ability to demonstrate a definite precipitin titre against antihemophilic globulin in the serum of each of these cases, it is suggested that the action of the anticoagulant against antihemophilic globulin is essentially that of an antibody-antigen reaction.

The presence in the blood of a hemophiliac of such an antibody, which manifests itself by inhibiting the substance antihemophilic globulin, could explain why these two patients were refractory to transfusion with whole blood or to injection of the concentrated globulin. It could also explain why blood from patients such as these inhibits the coagulation of normal blood.

On the basis of the evidence found, we present the following hypothesis as to the nature of the phenomena which developed in these two hemophiliacs. Each of these patients apparently lacks a globulin factor contained in normal blood or plasma which is necessary for normal coagulation. Because of this lack, repeated intravenous injections of normal whole blood, plasma, or antihemophilic globulin, all of which contain this missing globulin, result in "isoimmunization" with a "foreign" or missing protein. Antibodies of low titre are built up against this globulin and circulate in the blood. The presence of these antibodies is manifested as a circulating anticoagulant. After these antibodies become evident, injections of more blood or antihemophilic globulin in an attempt to supply the deficient globulin cause no beneficial effect on the coagulation mechanism because the active principle is inhibited by the antibodies. Likewise, when these antibodies are present, blood from one of these patients will exhibit an anticoagulant effect when added to normal blood *in vitro*, because the antibodies bind the globulin essential for normal coagulation.

The occurrence of these phenomena is accompanied clinically by findings which could be explained by the production of this immunologic response. Both of these patients seemed to react in the normal manner when blood was first given, but the

beneficial effect was very brief. Subsequent transfusions failed to alter the coagulation time; in fact, in the case of D. M. the coagulation time became progressively longer with repeated injections of blood and antihemophilic globulin. This refractory state was accompanied by the appearance of a demonstrable anticoagulant in the circulating blood.

It is highly possible that such a mechanism as described in these two patients may be the underlying factor in the development of a refractory phase in many hemophiliacs. Perhaps there are different degrees of hemophilia accompanied by different degrees of deficiency of the globulin fraction (or fractions) necessary for normal coagulation and individual variations in the response to injections of the globulin. Whether or not a complete lack of the globulin fraction is necessary for the development of an immunologic response to repeated injections of the deficient globulin is not known. Nevertheless, a certain number of hemophiliacs deficient in this globulin may be capable of developing antibodies against the globulin when it is given repeatedly. A refractory phase to further transfusion and the appearance of an anticoagulant in the circulating blood would result. Further search for the development of anticoagulants in hemophiliacs who become refractory to treatment with whole blood, plasma, or antihemophilic globulin is to be carried out to determine if such a mechanism may be more generally applicable.

#### SUMMARY AND CONCLUSIONS

1. Two cases of hemophilia are presented in whom the development of a circulating anticoagulant was detected. This anticoagulant was demonstrated in the whole blood, plasma, and serum of both patients.
2. Both patients became refractory to treatment with either fresh whole blood, plasma, or antihemophilic globulin (Fraction I of Cohn). In fact, the anticoagulant apparently made its appearance as a result of repeated transfusions or injections of antihemophilic globulin.
3. The anticoagulant in each case was shown not to inhibit any of the elements participating in the classical theory of clotting, i.e., prothrombin, thromboplastin, thrombin or fibrinogen. It was also demonstrated that this anticoagulant was not related to heparin in its mechanism of action.
4. Electrophoretic fractionation of the plasma was carried out, and it was found that the anticoagulant was associated with the gamma globulin fraction of plasma.
5. The demonstration of specific precipitin titres in the serum of each of these patients against antihemophilic globulin seemed to indicate that the mechanism of action of the anticoagulant was to inhibit the action of antihemophilic globulin. This was further substantiated by the *in vitro* inhibition of the ability of antihemophilic globulin to accelerate the coagulation time of ordinary hemophilic blood.
6. A hypothesis is presented to explain the appearance of the anticoagulant. It is believed that these two hemophiliacs are deficient in or lack antihemophilic globulin in their blood, and hence repeated injections of the globulin either in the form of whole blood, plasma, or Fraction I of Cohn results in "isoimmunization" against the injected globulin. The resulting antibodies inhibit any antihemophilic

globulin which may then be injected and hence explain the refractory state exhibited by these two cases. Blood from these patients containing these antibodies likewise exerts an anticoagulant influence when added to normal blood by the same mechanism.

7. A discussion of the implications of this phenomenon in the development of a refractory phase in hemophilia is presented.

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# THROMBOCYTIC ACROANGIOTHROMBOSIS (PLATELET THROMBOSIS OF THE CAPILLARIES, ARTERIOLES, AND VENULES)

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SINCE 1925 there have been described in the literature<sup>1-6</sup> 9 cases presenting acute purpura hemorrhagica, fever, thrombocytopenia in cases in which platelet counts were done, anemia, a rapidly progressive fatal course and the findings at necropsy of generalized arteriolar and capillary hyaline thrombi (tables 1-4). In most cases it has been stated that the thrombi are composed essentially of platelets and the disease has been considered to be a rare form of primary thrombocytopenic purpura. Recently, the suggestion has been made<sup>6</sup> that the disease is a distinct entity and not related to Werlhof's thrombocytopenic purpura or to any other known disease.

The first 8 cases occurred in females, the ninth in a male. We report 2 cases: one a white male; the other a Negress—the first to be described in a colored person.

## CASE REPORTS

*Case 1* (Case 10, tables 1, 2, and 3): A 34 year old, white male, C. W. H., Ensign, U. S. Navy, was admitted to a naval hospital, April 23, 1945, complaining of abdominal pain, weakness, ease of fatigue, anorexia, and constipation. He stated that he was well until three weeks prior to admission, when he first experienced slight nausea with severe epigastric pain. The pain developed about 30 minutes after eating and was relieved by further ingestion of food. A gastrointestinal series of roentgenograms shortly after the onset of symptoms was reported as negative. The patient carried on his duties as an officer aboard a small naval craft until the day prior to admission, when he reported to his medical officer because of increasing weakness.

There was no history of any familial disease. His past history was essentially negative except for an undetermined abdominal disorder at the age of 12 years that was followed by 8 months' bed rest. Review of the systems was noncontributory, except for nocturia two or three times for some years. There was no weight loss. He had been taking bismuth and Amphojel for several days prior to admission. Otherwise, there was no history of the ingestion of drugs. No history of allergy was obtained.

*Physical Examination:* The patient was a restless, apprehensive, well-developed young male. His temperature was 98.4° (F.), pulse 88, and respirations 20 per minute. Blood pressure was 104 systolic and 60 diastolic (mm. of Hg). The skin was pale and dry with poor turgor. Examination of the retinal fundi revealed recent, multiple petechial hemorrhages. The remainder of the examination was essentially normal except for slight, nontender enlargement of the anterior cervical lymph nodes and crepitant râles in the pulmonic area at the beginning of inspiration.

*Laboratory Data:* On admission to the hospital, the red cell count was 2.14 million per cubic millimeter and the hemoglobin 42 per cent. There were 13,700 white blood cells per cubic millimeter, 60 per cent of which were polymorphonuclear leukocytes, 10 per cent band forms, 1 per cent metamyelocytes, 1 per cent myelocytes, 27 per cent lymphocytes and 1 per cent monocytes. The platelet count was 88,000. The blood smear showed poikilocytosis, anisocytosis, and basophilic stippling. Clotting time was 3 minutes and bleeding time 11.5 minutes. Hematocrit was 18.5 per cent. Volume index was 0.87. Pro-

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The assertions and opinions contained herein are the private ones of the authors and are not to be construed as reflecting the opinions of the Navy Department.

thrombin time was considerably prolonged in comparison to a normal control specimen. Erythrocyte resistance to hypotonic saline showed hemolysis beginning at 0.42 per cent, being complete at 0.53 per cent. The control had a range of from 0.44 per cent to 0.30 per cent. A peripheral blood smear for plasmodia was negative.

TABLE 1.—*Clinical Features*

Case No.	Author & Year	Sex	Age	Symptoms and Signs	Physical Examination	Neurologic Findings	Duration
1	Moschowitz (1925) <sup>1</sup>	F	16	Weakness; joint pains; fever, 9 days	Pallor; petechiae; "café-au-lait tint"; fever	Paresis, left arm and leg; facial paralysis; bilateral Koernicke; coma	11 days
2	Baehr et al. (1936) <sup>2</sup>	F	9½	Listless, 6 months; hematuria, days	Pallor; fever; icterus; petechiae; splenomegaly; retinal hemorrhages	None mentioned	7 weeks
3	Baehr et al. (1936) <sup>2</sup>	F	18	Brownish pallor, weakness, 1 week	Icterus; petechiae; retinal hemorrhages; fever	Headache; clonic twitchings; irrational	2 months
4	Baehr et al. (1936) <sup>2</sup>	F	22	Urticaria, 1 month; purpura	Purpura; retinal hemorrhages; fever	Nausea; vomiting; delirium; stupor	9 days
5	Baehr et al. (1936) <sup>2</sup>	F	45	Arthritis, 1 year; U.R.I., 1 week; petechiae	Petechiae; icterus; retinal hemorrhages	Right hemiplegia; coma	2 weeks
6	Gitlow, Goldmark (Case 1) (1939) <sup>3</sup>	F	18	Influenza, 3 months; U.R.I., 1 week	Petechiae; fever; hepatomegaly; retinal hemorrhages; splenomegaly; palpable cervical nodes	Right hemiplegia; coma	14 days
7	Altschule (1942) <sup>4</sup>	F	50	Fatigue, 3 weeks; aches and pains, 4 days	Pallor; petechiae; fever; hepatomegaly; splenomegaly	Confusion; left facial weakness; delirium; mumbling speech	13 days
8	Bernheim (1943) <sup>5</sup>	F	33	Dermatitis; weakness, 2 weeks	Pallor; petechiae; shock; lid lag; enlarged thyroid; fever	Left facial weakness; hyperesthesias; tetanic convulsions	2 weeks
9	Trobaugh et al. (1946) <sup>6</sup>	M	24	U.R.I., malaise, 2 weeks	Fever; icterus; petechiae; cervical nodes	Restless; uncooperative stupor; coma	15 days
10	Fitzgerald et al. (1947)	M	34	Nausea; weakness; abdominal pain, 3 weeks	Pallor; petechiae; fever; retinal hemorrhages; cervical nodes	Restless; confused; coma; Cheyne-Stokes	5 weeks
11	Fitzgerald et al. (1947)	F(C)	27	Hives, 1 year; faintness; epistaxis; hematemesis; menorrhagia, 1 week	Pallor of mucous membranes; urticaria; hepatomegaly	Stupor; coma; Cheyne-Stokes respiration	1 week

The urine analysis was essentially negative. Stool examinations for occult blood were negative. The sedimentation rate was 30 millimeters in 60 minutes. The chest x-ray was normal and there was a normal electrocardiographic tracing. A blood culture failed to show any growth.

The icteric index was found to be 13.3. Qualitative tests on serum were negative for the presence of bromides or sulfonamide drugs. The presence of free HCl was demonstrated by gastric analysis.

*Course:* The patient was given ferrous sulfate by mouth and daily blood transfusions in 500 cubic centimeter amounts. There was a gradual increase in the red count to 3.3 million until 5 days after admission, at which time the count began to decrease until it was 2.5 million on the eighth hospital day.

TABLE 2.—*Hematological Data*

Case No.	R.B.C. (millions per cu.mm.)	Hemoglobin (percentage)	W.B.C. (per cu.mm.)	Platelets (per cu mm.)	Differential (percentage)
1	1.33	40	12,600		P/65
	1.12	40	19,000		No reticulocytes
2	1.5	28	13,700	25,000	Essentially normal in (2) (3) (4) (5) except for Reticulocytes, 2
	1.4	27	7,000	20,000	
		18			
3	1.7	35	11,000	60,000	Reticulocytes, 13
	2.2	44	17,000	50,000	Normoblasts, 55
	3.5	57	16,000	30,000	Myelocytes, 2
4	2.5	65	13,000	145,000	Myelocytes, 9
	1.4	29	28,000	20,000	Normoblasts, 18
5		40		Profound thrombocytopenia	Numerous myelocytes, normoblasts, and reticulocytes
6	1.4	25	Leucopenia	50,000	Reticulocytes 31-80
	2.0	46		100,000	High staff cell count Normoblasts very numerous
7	1.8	38	13,400	54,000	Reticulocytes 2.2-12.0
	1.9		21,000	11,000	P/80, L/15, M/4, E/1 Nucleated R.B.C.
8	2.0	38	9,900	Markedly reduced	P/57, L/17, E/8, B/2, M/1
	1.65	30	11,000		Myeloblasts 2, myelocytes 8, normoblasts 5
9	3.5	11.5 (Gms.)	8,700	Marked thrombocytopenia	P/54, L/23, M/17, E/4, B/2, blast/1. Reticulocytes 10
	2.18	36	9,200		
10	2.14	42	13,700	88,000	P/71, L/27, M/1
	2.5				Myelocyte 1
11	2.74	7 (Gms.)	14,900	Present	P/50, L/46, M/4 Normoblasts, polychromatophilia, poikilocytosis

The patient was very restless and often confused during waking hours and complained of dizziness. His temperature varied from 99° to 102° during the first 6 days of hospitalization and there was a corresponding variation in the pulse rate between 80 and 120 per minute. He had a normal temperature after his sixth hospital day. His respirations averaged about 22 per minute. Twelve days after admission the patient became unconscious, incontinent, developed Cheyne-Stokes respiration, and died about 5 weeks from the onset of his illness.

## AUTOPSY FINDINGS\*

*Gross:* Significant findings only are reported. Over the bridge of the nose and the right deltoid area there were ecchymoses; otherwise the skin shows only pallor. The pericardial cavity contained an estimated 150 cubic centimeters of serosanguineous fluid. The visceral pericardium showed diffuse ecchymotic areas, some small and discrete, but many confluent and extensive. The heart weighed 463 grams. The myocardium showed purpuric areas throughout and the endocardium adjacent to the pulmonary valve showed similar hemorrhages. Both lungs were similar and showed focal areas of atelectasis, congestion of the dependent parts, and edema and congestion of the tracheobronchial mucosa. The peritoneal

TABLE 3.—Additional Hematological Data

Case No.	Osmotic Fragility (hypotonic saline)	Bleeding Time (minutes)	Coagulation Time (minutes)	Clot Retraction	Tourniquet Test (Rumpel-Leeds)	Other Tests
1	0.8-0.19					Pallor of R.B.C. Blood culture negative
2	Normal	Irregularly prolonged in (2), (3), (4), (5)	Normal in (2), (3), (4), (5)	Markedly prolonged in (2), (3), (4), (5)	Promptly positive in (2), (3), (4), (5)	Blood cultures negative in (2), (3), (4), (5) Color index about 1.0 in (2), (3), (4), (5) (2) I.L. 23, V. Berg, 23 mg (3) V. Berg, 0.5-2.5 mc.
3	Also tested in one other—normal					
4						
5						
6	0.44-0.28	2-4	5	None at 24 hours	Negative	I.L. 14.2 Blood cultures negative V. Berg 0.6 mc. Wassermann negative
7		Usually over 30, once 11	2	Poor		I.L. 10 to 18 Hinton and Kahn negative
8		Over 30	23	None at 48 hours	Positive	Sed. rate 30 mm. I.L. 8
9	Hemolysis of 1% at 0.51; 10% at 0.41; 50% at 0.33; 75% at 0.26					I.L. 23 No cold agglutins
10	0.42-0.30	11.5	3			I.L. 13.3 Prothrombin time increased. Blood culture negative
11						Sed. rate 41 I.L. 25 Hinton negative

cavity showed firm adhesions between the loops of small bowel and an area of fresh hemorrhage over the surface of the right diaphragmatic leaflet. The liver was slightly enlarged. The spleen weighed 135 grams. The capsule was wrinkled and section showed congestion and prominence of lymphoid follicles. Many loops of small bowel were matted together by areas of organizing serosal hemorrhages. The gastrointestinal tract was negative otherwise.

Examination of the biliary ducts, gallbladder, pancreas, adrenals, kidneys, ureters, bladder, prostate, testicles, and aorta revealed no gross abnormalities. The bone marrow of the sternum and lumbar vertebrae was hyperplastic and oozed blood.

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The brain was normal in size, shape, and configuration. There was some thickening of the basilar meninges. There were diffuse small hemorrhagic areas in the gray matter of the cortex, throughout the basilar ganglia at the level of the superior colliculi, and in the pons and cerebellum.

*Histologic Examination:* Histologic sections, fixed in formalin and stained with hematoxylin and eosin, of the brain, spinal cord, heart, lungs, liver, spleen, adrenals, gastrointestinal tract, kidneys, prostate, testicles, lymph nodes, and bone marrow were available.

The most striking lesions, present to the greatest extent in the brain, heart, kidneys, and a lymph node, were thrombi of the arterioles, capillaries, and venules (figs. 1-5).<sup>\*</sup> The thrombi were made up of amorphous material staining a pink-red with hematoxylin and eosin stains. In some areas the vessels were filled with a finely granular matrix staining deeper than erythrocytes or hemoglobin (fig. 1). Occasionally, a small amount of the material occupied a portion of the vessel lumen. Often the adjacent endothelium of the vessels was normal. The vascular lesions of the brain, and a small number of lesions

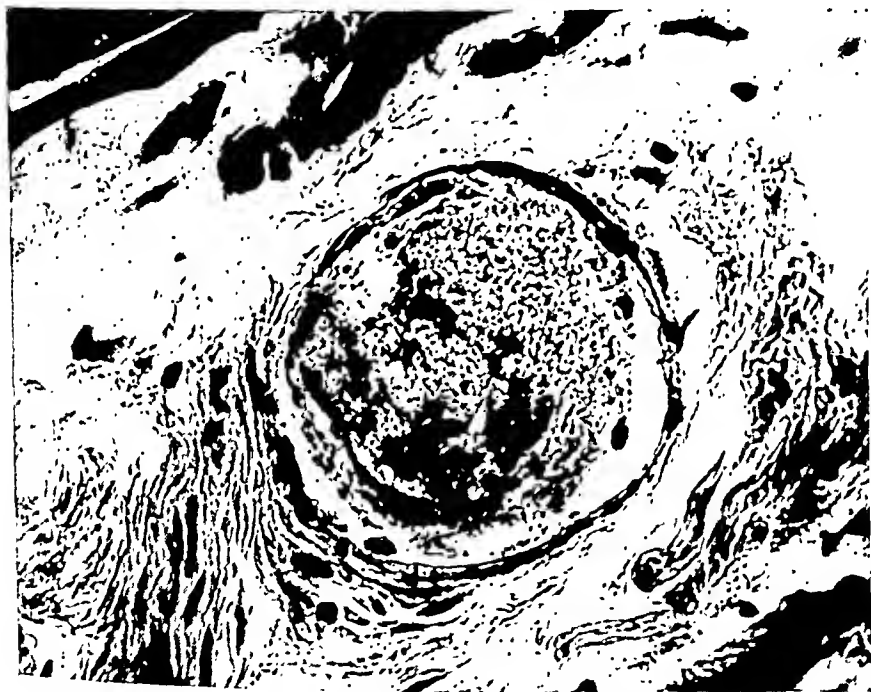


FIG. 1. MYOCARDIAL VENULE SHOWING EARLIEST LESION OF FINELY GRANULAR MATERIAL WITH FOCAL DEEPER STAINING AREAS. PHOSPHOTUNGSTIC ACID HEMATOXYLIN  $\times 1100$

elsewhere, showed the added feature of proliferating endothelium that narrowed the lumen in some vessels and occluded it in others (figs. 3-5). These lesions were most pronounced in the cerebral cortex but were present in moderate extent in the pituitary, choroid plexus, basal ganglia, pons, cerebellum, fourth ventricle, and spinal cord.

The arterioles, capillaries, and venules of the myocardium showed similar plugging, in the majority of cases by a combination of endothelial cells and hyaline material. There were focal myocardial necroses, fibrosis, and considerable epicardial hemorrhage. The kidney showed many lesions in the small cortical arterioles. These usually showed both hyaline material and endothelial proliferation. A glomerulus rarely showed a thrombus or a focal endothelial proliferation. One small lymph node showed the most

<sup>\*</sup> The photomicrographs were made by Mr. Leo Goodman, Boston City Hospital.

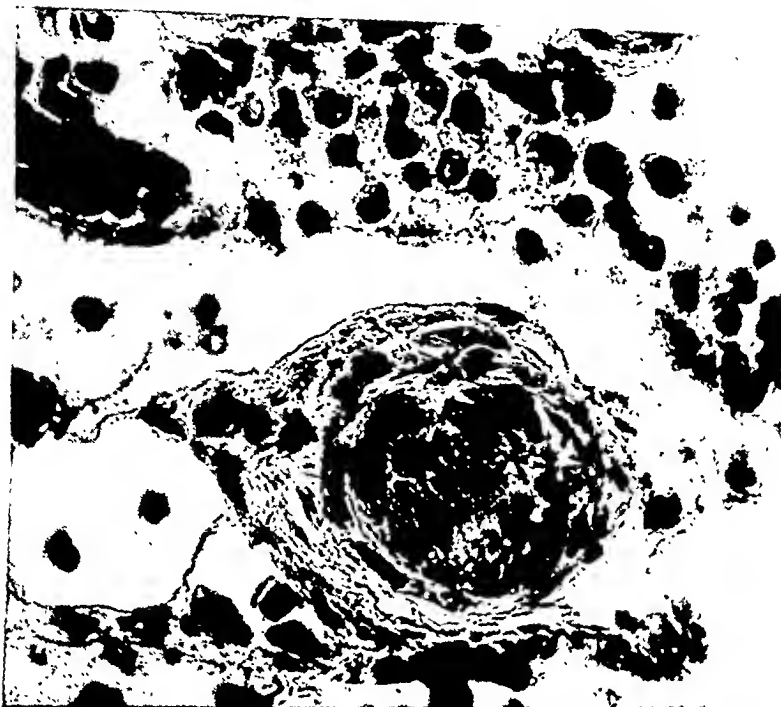


FIG. 2. VERTEBRAL MARROW ARTERIOLE SHOWING COARSE CLUMPS OF THROMBOTIC MATRIX WITH FOCAL ENDOTHELIAL PROLIFERATION. PHLOXINE METHYLENE BLUE X1000

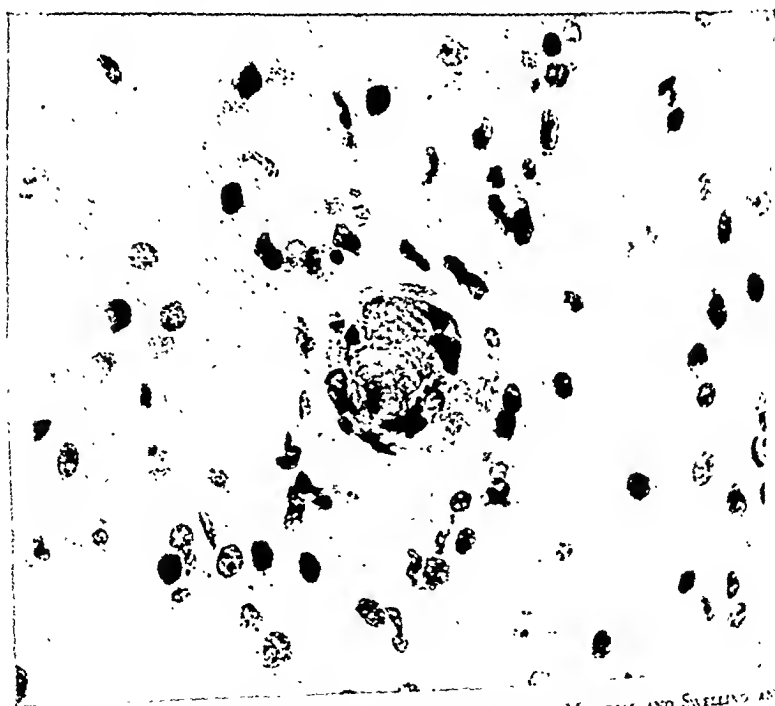


FIG. 3. CEREBRAL CORTEX VESSEL WITH COALESCENCE OF THROMBOTIC MATERIAL AND SWELLING AND PROLIFERATION OF THE ENDOTHELIAL. HEMATOXYLIN AND EOSIN X 540

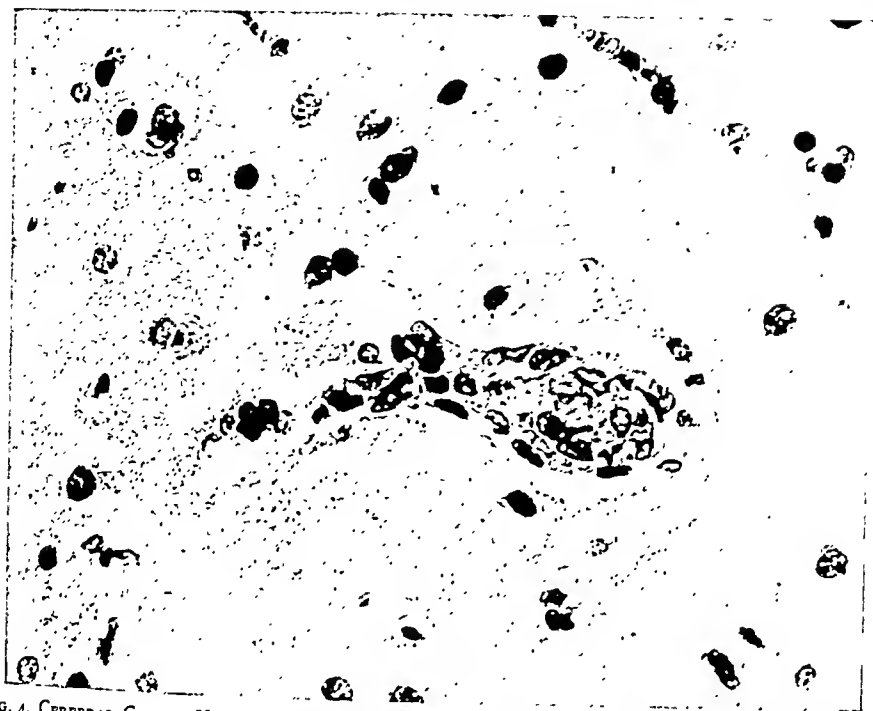


FIG. 4. CEREBRAL CORTX VESSEL WITH THROMBUS AND CONSIDERABLE ENDOTHELIAL PROLIFERATION. HEMATOXYLIN AND EOSIN  $\times 840$

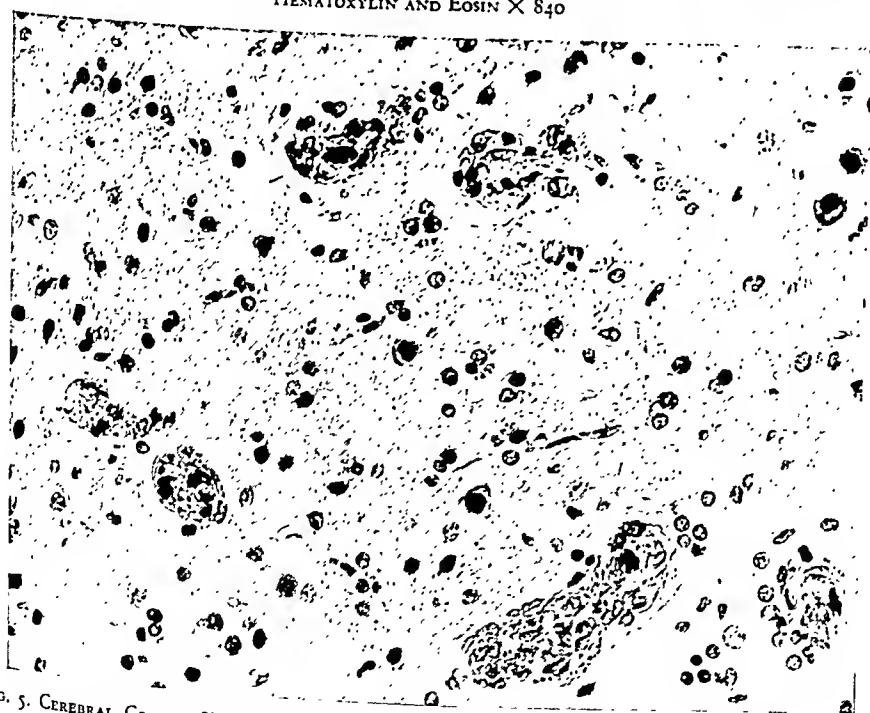


FIG. 5. CEREBRAL CORTX VESSEL SHOWING EXTENSIVENESS OF PROCESS, PREDOMINANCE OF ENDOTHELIAL PROLIFERATION, AND VARYING STAGES OF THE LESIONS. HEMATOXYLIN AND EOSIN  $\times 390$

extensive lesions with practically every arteriole and a few capillaries showing thrombi or a combination of thrombosis and endothelial proliferation.

In the small vessels of the portal areas of the liver, in the adrenals, spleen, bone marrow, and the mucosa and submucosa of the stomach there appeared similar lesions of either thrombosis or intimal proliferation, or both. A few thrombi were seen in the capillaries of the alveolar walls of the lungs. Megakaryocytes were also present in these blood vessels. The vertebral and sternal marrow showed hyperplasia of both the erythrocytic and granulocytic series. Megakaryocytes were apparently normal in numbers in some areas and appeared increased in others. A few capillary and arteriolar lesions were seen in both sternal and vertebral marrow.

*Anatomical Diagnoses:*\* Platelet thrombi with endothelial proliferation of the capillaries and arterioles of the brain, heart, lungs, liver, spleen, adrenals, gastrointestinal tract, kidneys, prostate, testicles, lymph node, spinal cord, and bone marrow; petechiae and ecchymoses of the face, pericardium, myocardium, lungs, peritoneum, small intestine, and brain; focal myocardial infarction; focal infarction of the cerebrum; hemopericardium; cardiomegaly; splenomegaly; hepatomegaly; hyperplasia of the bone marrow, vertebral and sternal; bronchopneumonia; atelectasis; tracheobronchitis; interstitial cell hyperplasia of the testicles; megakaryocytes in the lung capillaries.

*Case 2 (case 11, tables 1, 2, and 3):* D. R., a 27 year old, single Negress, was admitted to the Boston City Hospital November 28, 1946, by ambulance from her home because of deepening coma for the previous 12 hours.

The patient had a long history of admission to two hospitals for a variety of complaints. In 1941 the patient had gonorrhea, toxemia of pregnancy, and was delivered of a full-term stillborn fetus. In 1943 a Bartholin's cyst abscess was drained, and in 1945 she had an incomplete miscarriage. Extraction of abscessed teeth in the fall of 1945 was followed by rash ascribed to a sulfonamide given prophylactically with the extraction. In January 1946 she was hospitalized for chest and joint pains and showed urticarial lesions about the elbows and left arm. She was treated symptomatically and discharged free of symptoms in February 1946 with a diagnosis of rheumatic fever, acute.

The patient was followed in an outpatient clinic and had intermittent attacks of urticaria until her last admission to this hospital in November 1946. The attacks appeared to be precipitated by orange juice and contact with a dog. They were only slightly relieved by ephedrine or adrenalin in oil. In April 1946 the patient was given benadryl, 50 mg. every 3 hours, and in a few days her hives had disappeared. In July hives reappeared, benadryl treatment was reinstituted, and was followed shortly thereafter by a remission. In November urticaria reappeared and was generalized and the patient was given pyribenzamine for a short period before her death.

About a week after the reoccurrence of urticaria, the patient complained of faintness, suffocation, nausea, vomiting of blood, epistaxis, and a prolonged menstrual flow. Within a few days she became very weak, stuporous, and was admitted to the Boston City Hospital.

Physical examinations and laboratory tests during the patient's many outpatient visits and hospitalizations showed only a moderate anemia, a slightly increased sedimentation rate, and an electrocardiogram showed a P-R interval varying from 0.22 to 0.28-seconds.

Physical examination upon final admission to the hospital showed a well-developed, poorly nourished colored female in stupor. Blood pressure was 98 systolic and 76 diastolic (mm. mercury), pulse 90, and respirations 30 per minute. Temperature was 97.0° (F.). Pupillary reaction was equal, but sluggish. There was slight nuchal rigidity. Heart, lungs, abdomen, and extremities were negative. There was a macular, circumscribed rash with irregular margins over the chest, abdomen, back, and thighs. Knee jerks were absent, deep tendon reflexes sluggish; otherwise no focal neurological signs were present.

Laboratory tests showed (tables 2 and 3) a marked hypochromic anemia, with poikilocytosis, and polychromatophilia with normoblasts present in the smear. There was a moderate leukocytosis, an icteric index of 25, and normal blood sugar, nonprotein nitrogen and chloride values. A carbon dioxide combining power of 35 volumes per cent was found. Lumbar puncture findings were normal. Platelets were said to be "present" in the blood smear.

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\* We are indebted to Dr. Paul Klempner, who first identified the lesions of case 1 as those of platelet thrombi.

The patient was treated symptomatically, did not recover from coma, and died 6 hours after entry to the hospital. Death occurred about ten months after the onset of urticaria and vague generalized complaints, and about a week after acute symptoms appeared.

#### AUTOPSY FINDINGS\*

*Gross:* Significant findings only are listed. The visceral pericardium showed petechiae and ecchymoses. Section of the heart showed diffuse petechiae and hemorrhages throughout the myocardium, beneath the endocardium of both auricles and ventricles, and in the papillary muscles. Slight congestion and edema of both lungs was present. The spleen weighed 170 grams and the malpighian follicles were distinct. The liver weighed 1560 grams and was not remarkable. The combined weight of the kidneys was 340 grams and they were normal except for a few petechial hemorrhages in the pelvis. The right ovary contained two small cysts, one filled with blood, the other with serous fluid. The brain weighed 1210 grams and was not remarkable. The vertebral bone marrow appeared normal.

*Histologic Examination:* Almost all the smaller arterioles and many capillaries of the epicardium and myocardium were plugged by finely granular acidophilic material. Adjacent to these thrombi there was some endothelial proliferation in many vessels, but the majority showed little or none (figs. 1 and 2). These lesions were identical to the ones seen in case 1, except for less endothelial proliferation. Rare foci of early necrosis of muscle were present. Throughout all the myocardium and in isolated subepicardial areas there were areas of hemorrhage.

The lungs presented thrombi in the alveolar capillaries and these were frequently associated with proliferation of adjacent endothelium. Megakaryocytes were prevalent in the capillaries.

In the portal areas of the liver thrombi similar to those seen elsewhere were present. The spleen was characterized by marked congestion, a few foci of stem cells, normoblasts and myelocytes in the sinusoids. A few thrombotic lesions in the vessels of the stroma and in a rare arteriole of the follicles were present. In the pancreas were many vascular thrombotic lesions distributed throughout the organ in the acini and islets.

The branches of the interlobular arterioles in the kidney cortex contained many thrombi, some with slight endothelial proliferation. A rare glomerulus contained a typical thrombus in the tuft capillary. Some were seen in which the endothelium was apparently normal and in others there was associated increased cellularity of the capillary wall.

In the ovary, vaginal wall, uterus, esophagus, sympathetic ganglion, and a mesenteric node a few lesions were seen.

The vertebral marrow showed hyperplasia of both the erythropoietic and the granulopoietic series. The megakaryocytes appeared to be normal in number in some sections and increased in others. Many lesions in the arterioles and capillaries of the marrow were present (fig. 2).

The brain showed many thrombi, most of which were unaccompanied by endothelial proliferation although an occasional vessel was conspicuous because of the increased cellularity. The vessels of the cortex and basal ganglia were most involved. Foci where nerve cells had disappeared were replaced by astrocytes and microglial cells. Some petechial hemorrhages were present, usually about a thrombosed vessel.

*Anatomical Diagnoses:* Capillary and arteriolar thrombi with minimal endothelial proliferation of the heart, lungs, liver, spleen, kidneys, ovary, vagina, uterus, esophagus, sympathetic ganglion, lymph node, vertebral marrow and brain; focal myocardial necrosis; focal necrosis of the cerebrum; megakaryocytes in the lung capillaries; extramedullary myelopoiesis of the spleen; hyperplasia of vertebral marrow; petechiae and ecchymoses of the heart and kidney pelvis; healed pleuritis, left apex; splenomegaly.

#### REVIEW OF THE LITERATURE

The first case showing the specific lesions of this disease entity was studied by Moschowitz<sup>1</sup> in 1925. His patient (tables 1, 2, 3, and 4) was a 16 year old girl who had a 10 day febrile episode at home, where she complained of joint pains and weakness of the upper extremities. Later examination upon entrance to a hospital

\*A46-654, Mallory Institute of Pathology.

disclosed pallor, petechiae, leukocytosis, and a marked anemia. No platelet counts were performed. During her stay in the hospital the patient remained febrile, developed paresis of the left arm and leg, a slight facial paralysis, and a double Koernig reflex. On her fifth hospital day pulmonary edema was present, the patient became comatose, and she died two weeks after the onset of her illness. Autopsy disclosed hyaline thrombi in the terminal arterioles and capillaries. These were most abundant in the myocardium but also occurred in precordial fat vessels and in the liver, spleen, and kidneys. The thrombi appeared as hyaline masses partially or completely filling the lumina of small vessels and capillaries. In some places thrombi were invaded by fibroblasts and in other areas there was complete replacement of the hyaline material by proliferating fibroblasts. Vessels larger than terminal arterioles were not found to be involved. Moschowitz believed that the thrombi were composed of erythrocytes, but no differential staining of the lesions was reported.

In 1936 Baehr, Klemperer, and Schiffrin<sup>2</sup> reviewed the data on 4 additional cases that presented similar clinical and pathologic findings. They presented data (tables 1, 2, 3, and 4) showing that the disease is characterized by a rapidly progressive anemia, mild icterus, purpura hemorrhagica, and fever. Their patients were females. Leukocytosis, reticulocytosis and a marked thrombocytopenia were present. Clotting time was generally normal although clot retraction was poor. Bleeding time was prolonged. Transfusions did not appear to be of much assistance. Splenectomy was performed on 1 patient, but the patient died shortly after operation. Death occurred from nine days to two months from the onset of symptoms. Necropsy revealed multiple diffuse hemorrhages into viscera and the presence of thrombi in capillaries and arterioles. Some of the thrombi were acellular while others were undergoing various stages of endothelialization. Very little evidence of necrosis was present distal to these thrombi. These authors showed that the thrombi contained no erythrocytes, hemoglobin, or any significant amount of fibrin. They believed that the thrombi were composed of platelets. Gitlow and Goldmark (case 1) in 1939,<sup>3</sup> Altshule in 1942,<sup>4</sup> and Bernheim in 1943<sup>5</sup> reported similar cases, also in females. Bernheim's case was the first to show ischemic necrosis in the brain and this author also found megakaryocytes in the lung. Trobaugh et al.<sup>6</sup> described, in 1946, the first case to be reported in a male, showed that no collagen or fibrils were present in the thrombi, and stated that the disease was distinct and not a variant of essential purpura hemorrhagica.

Our case 1 was the second to be described in a male and was characterized by diffuse extensive lesions of the brain with focal areas of hemorrhage, necrosis, and gliosis, most pronounced in the cortex. Case 2, a Negress, was the first case described in a colored person. This case also had extensive brain lesions and clinically was of interest because of her history of urticaria and treatment for many months by two new antihistaminic drugs—benadryl and pyribenzamine.

#### SUMMARY OF PATHOLOGIC FINDINGS (TABLE 4)

*Gross:* Splenomegaly has been listed in all autopsy protocols. The weights were given in 7 cases and vary from 165 grams to 540 grams, averaging about 300 grams. Infarcts were present in 5 cases.

Areas of petechial hemorrhages or ecchymoses have been noted in 10 cases. Skin, serosal surfaces, and the heart have been the most common areas involved. The bleeding has been focal and generally slight, but in our case 1 there were 150 cc. of serosanguinous fluid in the pericardial cavity. No massive intracranial bleeding has been reported in any of the 5 cases in which the brain was examined.

Hepatomegaly was recorded in 3 cases, but the weight of the organ was given in only 1—2000 grams in an adult. One autopsy revealed a nonbacterial thrombotic endocarditis of the mitral valve.<sup>2</sup> Enlarged axillary, mesenteric, and para-aortic nodes were present in 1 case.

TABLE 4.—*Autopsy Findings, 11 Cases*

	No. of Cases
<b>A. Gross:</b>	
Splenomegaly.....	11
Petechiae or ecchymoses.....	10
Hepatomegaly.....	3
Enlarged nodes (axillary, mesenteric, and para-aortic) ....	1
Nonbacterial thrombotic endocarditis.....	1
<b>B. Microscopic:</b>	
Platelet thrombi—capillaries, arterioles, or venules	
Heart, kidneys, liver.....	11
Pancreas.....	10
Adrenal.....	9
Spleen.....	6
Brain.....	4 (5 examined)
Infarcts (focal, small)	
Heart.....	7
Spleen.....	5
Liver.....	4
Brain.....	3
Kidney.....	2
Adrenal.....	1
Hyperplastic marrow.....	9
Megakaryocytes in lung.....	4
Intralobular edema of liver.....	3
Hemosiderosis.....	3

*Microscopic:* Platelet thrombi were present in the heart, kidneys, and liver of every case. In 10 cases the pancreas was involved and in 9 the adrenals showed the lesion. The spleen showed thrombi in 6 cases, and in 4 of the 5 cases in which the brain was examined lesions were present. Small, focal infarcts were present in the hearts of 7 cases, in the spleen of 5, in the livers of 4, in the kidneys of 2 cases, and once in the adrenals. Almost all organs of the body have been involved in the composite group of cases.

The central nervous system has been examined in 5 cases. One case showed no thrombi or parenchymal damage.<sup>3</sup> The observation that the patient developed a right hemiplegia terminally was of interest. One case presented vascular thrombi,

but no parenchymatous damage was reported.<sup>6</sup> Thrombi were associated with focal ischemic necrosis of the cortex in 1 patient.<sup>5</sup> Both of our cases showed cerebral damage associated with vascular thrombi. Focal hemorrhage, necrosis, and gliosis occurred throughout the brain but were more prominent in the cortex.

The bone marrow was described in 9 cases and was said to be hyperplastic in all. The megakaryocytes are usually not described. In 1 case they were said to be present in the usual numbers and appeared to be consistent with old megakaryocytes. Both of our cases showed some areas with an apparently increased number of megakaryocytes and other areas where they were normal. We were unable to detect in our autopsy material any difference in the megakaryocytes of our cases and those of 6 autopsied cases of primary thrombocytopenic purpura.\* However, the rapid disintegration of the cytoplasmic detail of megakaryocytes in autopsy material is well known, and biopsy or aspiration of marrow will probably have to be used to determine whether platelets are being properly produced in the megakaryocytes in this disease.<sup>7</sup>

The presence in the lungs of megakaryocytes has been reported in 2 previous cases and was conspicuous in both of ours. These findings have been reported in Hodgkin's disease, aplastic anemia, and various blood dyscrasias. We also noted the giant cells in each of 6 cases of idiopathic thrombocytopenic purpura that were reviewed.\*

Hemosiderosis was present in 3 cases. The spleen was involved twice, once in association with the liver, and in 1 case the kidneys alone were involved. Three cases showed an intralobular hepatic edema.

*Studies of Thrombi:* In an attempt to determine the nature of the thrombi special staining of tissues was carried out. The tissues of case 1 had been fixed in formalin, and those of case 2 were fixed in Zenker's fluid and in solution of 10 per cent formalin. Hematoxylin and eosin were used as routine stains in case 1 and phloxine-methylene blue in case 2. All staining was carried out through standard procedures.<sup>8</sup> Weigert's fibrin stain, Mallory's aniline blue connective tissue stain, phosphotungstic acid hematoxylin stain, Giemsa stain (Wolbach's modification), van Gieson and elastic tissue stains, iron stain, hemofuscin and hemoglobin<sup>9</sup> stains, and reticulum stains were done on representative sections. Brain sections were fixed in formalin and stained with cresyl violet, toluidin blue, and hematoxylin and eosin.

The earliest lesion that we have recognized (fig. 1) has been an amorphous finely granular precipitate in capillaries, arterioles, or venules that takes a deeper stain with phloxine and eosin dyes than do erythrocytes, hemoglobin, or plasma proteins. This material has usually filled the lumen of the vessel, but occasionally it was deposited along a small segment of vessel wall and projected only slightly into the lumen. At this stage there was very little or no swelling or proliferation of the vascular endothelium. Some areas in the material stained heavily and appeared to be discrete masses suggesting that cells were trapped in the thrombus, or that consolidation of the substance had occurred. A more common lesion, apparently a

\* From the autopsy and surgical files at Mallory Institute of Pathology from 1932 through 1946.

later one, was the coalescence of the finely granular material into large coarse clumps of deeper staining matrix. Associated with this was a swelling of the endothelium lining the vessel (fig. 2).

A later stage of the process, we believe, was the coalescence of granular material into a hyaline thrombus that stained evenly and deeply with the acid dyes. Usually this was associated with focal endothelial swelling and proliferation with the cells aligned about the periphery of the thrombus (fig. 3).

Another type of lesion, seen most frequently in the brain, was one that we interpret as a further stage of the process (fig. 4). The thrombi were compact, hyaline, stained deeply, and were accompanied by considerable endothelial proliferation. In some regions this endothelial reaction was adjacent to thrombi, yet in the same vessel, a few micra away where no thrombotic material was evident, there was such endothelial swelling and proliferation that the lumen was markedly narrowed or obstructed.

The lesions that we interpret as earliest (figs. 1 and 2) were most numerous in case 2, one of only a week's duration. The lesions showing endothelial proliferation as well as vascular thrombi (figs. 3 and 4) were more common in case 1, which was of about 5 weeks' duration. However, even in the brain of case 1, in areas of extensive involvement one could find some areas showing all stages of the process (fig. 5). In case 2 a few vessels could be found showing much endothelial proliferation. In view of these findings and the occasional occurrence of laminated thrombi where endothelial cells were interspersed between the layers of platelets, we believe, with others, that the process is an intermittent one.

With Mallory's aniline blue stain the thrombi stained either a lavender or pale red, but no fibrillar elements were present. Phosphorungstic acid hematoxylin stained the thrombi a pale brown, but again no fibrils were present. A strand of occasional fibrin stained blue. Weigert's stain for fibrin colored the thrombi a dull gray and only rarely were a few strands of blue fibrin seen in the thrombi. Weigert's and van Gieson's stains revealed, respectively, no elastic fibers or connective tissue in the thrombi. Turnbull's blue stain for iron revealed the thrombi to be a dull red and no blue granules of iron could be seen. A reticulum stain showed a slight increase in reticulum in those capillaries of the brain with endothelial proliferation. Giemsa stain (Wolbach's modification) showed the fused thrombi to be purple or bluish. The finely granular material in the earlier lesions stained a dark blue, but no red color was apparent. Even in normal vessels with erythrocytes and leukocytes no red and blue granular material similar to that present in the usual peripheral blood smear was seen. In these normal vessels finely granular material, possibly platelets, stained dark blue as did the finely granular material of the thrombi. The Giemsa stain did not reveal bacteria or inclusion bodies. Hemoglobin stains<sup>9</sup> showed the thrombi to be a red or brownish red in contrast to the olive green or green of erythrocytes and granular material in uninvolved vessels.

Differential staining has shown, we believe, that the thrombi are not composed of erythrocytes, hemoglobin, hemosiderin, leukocytes, collagen, or fibrin (though slight amounts of the last may be present). No bacteria nor inclusion bodies were

found in the vessels. We believe that the thrombi are masses of platelets despite the fact that definite red and blue material could not be seen. The well-known rapid disintegration of platelets in autopsy material precludes their absolute identification even in normal vessels. Chiefly by excluding the substances known to be present in the usual vascular thrombus, the conclusion is reached that the thrombi in this disease entity are composed of platelets.

*Differential Diagnosis:* Because of the similarity of clinical findings and course, it is obvious that idiopathic thrombocytopenic purpura (Werlhof) is the disease most likely to be considered in these cases. To determine if platelet thrombi were found in Werlhof's disease Baehr et al. re-examined the lesions in 10 autopsied cases (7 acute and 3 chronic) of this disease.<sup>2</sup> They failed to find a single lesion showing platelet thrombosis. One of us (P. J. F.) has examined the histologic sections of 6 autopsied cases of primary thrombocytopenic purpura that had been well studied clinically.\* Three of these were acute and 3 chronic. No vessel showing any thrombus similar to those present in cases 1 and 2 was observed. Histologic sections of 12 spleens removed from patients with primary thrombocytopenic purpura were also examined. Seven of these were of the chronic variety and 5 acute. No vessel showing platelet thrombosis was observed in any of these. In spite of the similarity of many of the clinical findings in the two diseases, we believe that our cases belong to an entity whose platelet thrombi distinguish it from primary thrombocytopenic purpura (Werlhof).

The considerable endothelial proliferation of the cerebral vessels brings to mind the lesions of the rickettsial group, and the diagnosis of one of these was considered when the slides of case 1 were first seen. Furthermore, it has been reported that platelet thrombi are found in scrub typhus (tsutsugamushi fever) by Allen and Spitz<sup>10</sup> in their thorough study of the disease and its comparison with epidemic (louse-borne) typhus and Rocky Mountain spotted fever. These authors showed that platelet thrombi could be found in the eschar, the primary lesion of scrub fever, and also in the macule, its rash. Infrequently the glomerular tuft capillaries of the kidneys or the septal capillaries of the lungs showed platelet thrombi. However, these authors found no platelet thrombi in the brain and they point out that, contrary to the usual teaching, there is a "scarcity of histologically evident vascular damage in scrub typhus." Epidemic typhus and Rocky Mountain spotted fever show much more vascular endothelial proliferation, in general, but these cases are accompanied by arteritis or phlebitis and no evidence of platelet thrombi has been reported. All three of these rickettsial diseases frequently showed some pancarditis, interstitial pneumonitis, "typhus nodules," or microinfarcts in the brain. They also are characterized by certain responses believed, by Allen and Spitz, to be allergic, i.e., fibrinoid necrosis of collagen, necrosis of lymph nodes and spleen, a characteristic cellular infiltrate with predominance of a basophilic macrophage, and an acute, diffuse glomerulonephritis. The multiplicity of lesions should differentiate these diseases from diffuse platelet thrombosis. Allen and Spitz also state that the brain lesions of toxoplasmosis and malaria "granulomas" resemble the

\* From the autopsy and surgical files at Mallory Institute of Pathology from 1932 through 1946.

findings in Rocky Mountain spotted fever, while Chagas disease resembles those of epidemic and scrub typhus. Rarely these might have to be considered, but the presence of the specific parasite in the lesions solves the diagnostic problem.

Periarteritis nodosa was considered clinically in case 2 in the belief that the patient might have become sensitive to benadryl. However, the restriction of lesions to the smallest arterioles, capillaries, and venules and the absence of inflammation in the walls of the vessels are in marked contrast to the panarteritis of arteries and medium sized arterioles seen in periarteritis nodosa.

Acute disseminated lupus erythematosus has been mentioned<sup>3</sup> as being closely related to the group of cases under discussion. Klemperer et al.<sup>11</sup> describe in lupus erythematosus disseminata occasional "hyaline thrombi" in the glomerular tufts of the kidney. However, these authors, in their extensive studies of the disease, have shown that a characteristic feature is the diffuse degeneration of collagen. The "hyaline thrombi" are considered to be extreme forms of degeneration of basement membrane collagen similar to the "wire loops" described by them. Thus, of most importance in differentiating the two diseases would be the lack of platelet thrombi and the presence of diffuse fibrinoid degeneration of collagen.

Endothelial proliferation of capillaries and arterioles is not uncommon in brain lesions such as hemorrhage, thrombosis, tumors, and the encephalitides, but in none of these are platelet thrombi prominent and the vascular changes are secondary to the easily distinguishable primary process.

Certainly, any blood dyscrasia showing relatively normal leukocyte and differential counts with an anemia, thrombocytopenia, and bleeding tendencies would cause some difficulty in diagnosis. Symptomatic purpura of various types, aleukemic leukemia, aplastic anemia, pernicious anemia, and various types of neoplasia might simulate the disease occasionally. Bone marrow aspiration or biopsy should decide the diagnosis in most cases.

#### DISCUSSION

Thrombotic lesions of the type seen in the described disease are unique and their occurrence has aroused much discussion as to their nature and pathogenesis. The consensus, at present, is that they are composed of blood platelets and our studies are in conformity with this belief.

If it can be decided that the material of the thrombi is platelet in origin, then a greater problem is the explanation of the pathogenesis of the disease. For the ordinary vascular thrombus is made up, largely, of erythrocytes enmeshed by fibrin and platelets, and normally platelets do not spontaneously precipitate and form pure platelet thrombi. A study of platelet agglutination would seem to be a profitable field of exploration in this disease, especially in view of Ollgaard's demonstration<sup>12</sup> of the marked differences in platelet agglutination factors in febrile diseases, bed rest, and postoperatively. However, his technic of platelet agglutination has not been reported in this type of case. The only reported study on platelet agglutinins has been done on postmortem blood serum by Bernheim, who could not demonstrate in her case the presence of agglutinins for platelets of the same blood group as her patient.

Recent work by Copley and associates<sup>18</sup> has shown that platelet agglutination may occur independently of the usual mechanisms of blood clotting. They showed that whereas purified globulin fractions, purified prothrombin and thrombin, and plasma containing heparin in high concentrations all caused agglutination of platelet suspensions, purified fibrinogen never caused agglutination. They concluded that platelet agglutination was not brought about by fibrin formation—an important link in the chain of ordinary vascular clotting—and that the two processes were governed by different mechanisms. We have been unable to find any reported studies on abnormally formed globulin fractions or other substances that might give rise to platelet agglutination.

Because of the absence of studies of platelet agglutination in these cases and the presence of considerable endothelial proliferation in the thrombi, it has been postulated that the initial lesion in the disease is damage to the endothelium. The presence in some tissues of endothelial proliferation in the absence of platelet thrombi further strengthens the possibility of the platelet depositions being secondary to endothelial damage. Trobaugh *et al.*<sup>6</sup> reported endothelial mitoses in vessels devoid of thrombi. However, there is apparently no definite relationship between the amount of endothelial proliferation and the platelet thrombosis unless it be a matter of the duration of the disease process. In case 1, of five weeks' duration, the cerebral vascular proliferation was considerable. In case 2, of only a week's duration, the brain showed more endothelial proliferation than other organs, but even in these cerebral vessels platelet thrombi were predominant. Both of our cases, in general, were found to have overwhelmingly more examples of thrombosis without endothelial proliferation than the reverse, especially in case 2, where extracerebral endothelial proliferation was uncommon. However, occasionally endothelial proliferation without apparent platelet thrombi was present. In agreement with other observers, we found varying stages of lesions even in the same high power field and interpret this as evidence of the intermittent nature of the process. With the evidence available it does not appear possible to decide, on morphologic findings, whether endothelial damage is primary or secondary.

#### CLINICAL ASPECTS (TABLE 1)

In all patients there has occurred abnormal bleeding and in 10 cases it has occurred in the form of petechiae or ecchymoses. Usually there have been vague symptoms for weeks to months of malaise, weakness, or joint pains. Urticaria has been prominent in 3 cases and 3 others were said to have had "upper respiratory infection" before the acute onset of symptoms. Progression of symptoms or the occurrence of petechiae or hematuria has been the acute factor hospitalizing patients. Physical examination usually revealed pallor, frequently icterus or a "café-au-lait" appearance (5 cases), and petechiae or ecchymoses. Retinal hemorrhages have been mentioned in 6 cases, and only one examination of the retina was listed as negative. Splenomegaly has been noted in 3 cases, one of which also had hepatomegaly. Hepatomegaly was noted in 2 other cases unaccompanied by splenomegaly. Enlarged cervical lymph nodes were noted in 3 patients. Moderate elevation of temperature was recorded in 10 cases.

One patient entered the hospital in stupor and another in semicoma. Ten of the 11 cases gave evidence at some time in the disease, usually terminally, of central nervous system involvement. Headache, confusion, delirium, reflex changes, hyperesthesias, stupor, coma, convulsions, facial weakness, and hemiplegia have been recorded.

The course of all reported cases has been progressively downhill with death usually occurring within a few weeks of the onset of acute symptoms.

#### HEMATOLOGIC FINDINGS (TABLES 2 AND 3)

All patients have shown a marked anemia and, when mentioned, the color index has been said to be "about 1" (table 2). Leukocyte counts have usually been within normal limits or have shown a slight increase. One case showed a count as high as 30,000 per cu. mm. and 1 case was said to have had "leukopenia." The differential counts have shown polymorphonuclear leukocytes, lymphocytes, monocytes, eosinophils, and basophils to be within normal limits usually. In 1 case a "high staff" count was present and frequently a few myelocytes were noted. Myeloblasts and "blast" cells have been found occasionally. As might be expected with such a marked anemia, the erythrocytes have shown considerable abnormalities. Polychromatophilia, poikilocytosis, anisocytosis, reticulocytes, and normoblasts have been present.

In the first case studied no platelet count was reported. In one of our cases the only remark about platelets states that they were "present." In 3 cases it was noted that "the platelets were reduced," there was a "marked thrombocytopenia," and the third showed a "profound thrombocytopenia." In the 6 patients whose counts are recorded the lowest was 11,000 per cu. mm., the highest was 145,000, and the average value of 15 counts done on the 6 patients was 57,000 per cu. mm. Unfortunately, as in many of the other procedures, the normal values for the individual laboratory reporting the results are not given.

Erythrocytic fragility to hypotonic saline has been tested in 6 cases and has been essentially normal (table 3). Bleeding time has been reported in 8 cases. In 1 case it was within normal limits. In 4 cases it was stated to be "irregularly prolonged." Eleven minutes is given for another case, and 2 were over 30 minutes. Clotting time was performed in 8 cases and was normal in 7. One report gave 20 minutes, but the method used was not mentioned. Clot retraction was mentioned in 7 cases. It was stated to be "markedly prolonged" in 4, "poor" in 1, "nonretractile in twenty-four hours" in another, and "nonretractile in forty-eight hours" in the seventh. A tourniquet test was reported in 6 cases. It was "negative" in 1, "positive" in another, and "promptly positive" in 4 cases.

Trobaugh et al. decided from their studies that their patient had hemolytic anemia and they consider this to be a feature of the syndrome. In most cases the data are not sufficient to decide the matter as to whether the anemia is hemolytic or not. Neither of our cases showed any hemosiderin deposits in the spleen, liver, or bone marrow. Nor were the splenic changes said to be characteristic of the disease present. Osmotic fragility tests have been essentially normal, but it is well known that they may be so in hemolytic anemia.

Blood cultures were reported as negative in 7 cases. Icteric indices were performed on 6 cases and ranged from 8 to 25. Van der Berg quantitative analyses showed values of from 0.5 to 2.5 mg. Urobilinogen was tested for in 1 case and was present in a 1:10 dilution of urine. Hinton, Kahn, and Wassermann serologic tests were negative. Cold agglutinins were searched for in 1 case, but no abnormal titers found.

*Other Laboratory Tests:* Urine examinations were reported in 10 cases: 2 were negative, 1 showed gross hematuria, and 7 showed microscopic hematuria. Various determinations for blood nonprotein nitrogen, urea, uric acid, creatinin, sugar, calcium, phosphorus, and phosphatase have been within normal limits. Two determinations of total blood cholesterol were reported—one slightly above and one slightly below normal limits. In both the cholesterol esters were decreased. Stool cultures were negative for pathogens. A total protein determination in 1 case was low (5.6 Gm.) but the albumin-globulin ratio was normal. Lumbar puncture has been reported as negative in 2 cases. Electrocardiograms and roentgenograms of the chest have been essentially negative.

#### ETIOLOGY

Moschowitz, influenced by Flexner's work showing that old erythrocytic or compact erythrocytic thrombi give a hyaline appearance in histologic sections, believed that the thrombi observed by him were composed of erythrocytes. He thought that some powerful poison with agglutinative and hemolytic properties was responsible for the strange changes found.

Baehr et al. considered the disease a form of idiopathic thrombocytopenic purpura. They suggested that this disease was a product of a sensitivity phenomenon involving vascular endothelium. They believed that in the usual case of thrombocytopenic purpura, platelets escaped to the extravascular tissues through damaged endothelium. In the rare cases of platelet thrombi there was intravascular agglutination of platelets with subsequent withdrawal of these elements from the circulating blood with the symptoms of thrombocytopenia. They pointed out that the vascular lesions resemble those in the Shwartzman phenomenon except for their location in the venules in the latter reaction.

In relation to the factor of sensitivity, 3 patients had urticaria. One of these, our case 2, had severe intermittent attacks of hives for one year prior to death. The first attack was associated with the administration of a sulfonamide and was believed to be directly related to the use of the compound. The patient was treated with benadryl and later pyribenzamine for repeated attacks of urticaria said to have been precipitated by agents such as orange juice and the presence of a dog. In view of the recent emphasis upon the possible role of hypersensitivity to sulfonamide compounds and other agents in the production of periarteritis nodosa,<sup>11</sup> one cannot avoid the conjecture that an analogous mechanism might be present in these cases and that it produces platelet thrombi in response to various sensitizing agents—antihistaminic drugs, or some sulfonamide. It is of interest that recent reports on benadryl<sup>15-18</sup> have emphasized the toxicity of the drug and emphasis has been laid on the involvement of the central and peripheral nervous systems. It is also of note

that many of the manifestations of the rickettsial diseases, in which platelet thrombi are occasionally seen, are said to be allergic in nature.<sup>10</sup> However, no similar lesion has been described in the toxicologic studies of benadryl<sup>19</sup> or of pyribenzamine,<sup>20</sup> and none of the other cases were known to have received antihistaminic or allied drugs. For these reasons we do not believe that we can decide from available evidence whether or not these drugs had a causal relationship to the disease.

The lack of inflammatory exudate in the lesions and the absence of bacteria or inclusion bodies in the endothelium of involved vessels speak against an inflammatory factor. Experimentally, Kielanowski and Selzer are said to have found platelet thrombi in the vessels of rabbits receiving intradermal injections of *Escherichia coli* culture filtrate.<sup>21</sup> However, these reactions are accompanied by a polymorphonuclear infiltration not seen in human cases. Injection of colloidal materials into animals will produce agglutination of platelets, platelet thrombi, and an extreme thrombocytopenia.<sup>7</sup> Whether such a phenomenon is at work in this disease cannot be decided at present.

It is patent that the cause of this disease is unknown. We do not believe that it is a form of periarteritis nodosa, lupus erythematosus disseminatus, or idiopathic thrombocytopenic purpura. With Trobaugh et al., we believe that it is a distinct entity.

#### DIAGNOSIS

No case has been diagnosed correctly prior to death. Because of the bleeding tendencies and low platelet count the usual diagnosis has been one of thrombocytopenic purpura, either primary or secondary. In our second case the previous history of headaches, and the stupor led to an admission diagnosis of brain tumor.

The rapid progress of the disease and the neurologic signs are well-known possible sequelae also in primary or secondary thrombocytopenic purpura, so that either event is not necessarily diagnostic. Anemia and icterus may also occur with purpura hemorrhagica. However, the syndrome of an acute febrile illness, thrombocytopenia, purpura or any bleeding tendencies, anemia, icterus, and progression to central nervous system involvement should suggest the possibility of capillary and arteriolar thrombi. A bone marrow biopsy showing the platelet thrombi in capillaries or arterioles would be diagnostic. In view of the presence of the lesions in the sternal marrow in our case 1 and in the vertebral marrow of case 2 it is believed that sternal biopsy should reveal the diagnostic thrombi in most of the cases. Although no reports of the presence of thrombi in skin lesions have been found, and sections of skin examined in case 2 showed no lesions, possibly they are present and could be diagnosed by biopsy. Spleens removed from patients with thrombocytopenic syndromes should be carefully examined for the presence of vascular thrombi as the organ has shown the specific lesion in 6 cases of the 11 examined.

#### TREATMENT

Blood transfusions, intravenous glucose and saline, intensive vitamin therapy, and supportive measures apparently do not arrest the course of the disease. Splenec-

tomy was attempted in 1 case, but the patient died so soon after operation that this type of therapy could not be properly evaluated.

In view of the fatal progression of all cases known at present, the uncertainty regarding etiology, and the known dramatic results in some cases of idiopathic thrombocytopenic purpura following splenectomy, the latter procedure might well be attempted if the diagnosis were made and if the patient did not respond to medical treatment.

#### NOMENCLATURE\*

Inasmuch as the disease appears to be an entity and has been described by cumbersome titles, a descriptive, shorter name would be appropriate. Since we are forced to conclude, with the data available at present, that the essential feature of the disease is a platelet thrombosis of the terminal arterioles, capillaries, and possibly venules we propose the name "thrombocytic acroangi thrombosis."

This term would localize the lesion (acro—terminal, angio—vessel), state the nature of the process (thrombosis—plugging of a blood vessel by a clot), and identify the chief constituents of the lesion (thrombocytes—platelets).

#### SUMMARY

It is believed that there is a definite disease syndrome characterized clinically by fever, marked anemia, purpura, thrombocytopenia, central nervous system involvement, and a progressive fatal course of a few weeks' duration. Histologically the presence of platelet thrombi in the capillaries, arterioles, and venules is pathognomonic. Only by histologic demonstration of the platelet thrombi can the disease be definitely differentiated from idiopathic thrombocytopenic purpura.

The data of 9 previously reported cases of this rare disease are summarized and 2 additional cases reported.

Diagnosis of the disease probably can be made by bone marrow biopsy or, possibly, by skin biopsy. If a case be diagnosed and the patient is not responding to conservative treatment it is suggested that splenectomy be considered.

Spleens removed for thrombopenic diseases should be examined for platelet thrombi in the possibility that unrecognized cases of the disease have been treated by this procedure. Proper evaluation of splenectomy in this disease might be aided by follow-up study of any cases showing platelet thrombi.

The name "thrombocytic acroangi thrombosis" is suggested for this disease.

#### ADDENDA

##### Case 3

Since this article was submitted, a third case has been studied (M.I.P., A-47-384). A 24 year old white male printing press operator of the Jewish religion entered the Boston City Hospital April 30, 1947. Three weeks prior to entry the patient had pains in both knees. One week prior to admission he had chills, fever,

\* We are indebted to Dr. Robert M. Green, of Boston, for his assistance in determining the terminology of this disease.

backache, and abdominal pain. He was treated by a physician with aspirin but upon recurrence of symptoms, 2 days prior to admission, he was given a sulfonamide. Vomiting occurred 2 hours after the initial dose, but subsequently at least 12 tablets were taken. One day prior to admission a macular rash appeared over the face and neck, his urine was red, and epistaxis occurred. Sulfonamide administration was stopped but the urine remained red. His physician advised hospitalization and thought the patient had sulfonamide toxicity. The patient stated that he had had rheumatic fever at 12 years of age and was kept in bed for 3 weeks. At 18 years he was rejected by his draft board because of cardiac murmurs. One year prior to admission he had taken 12 tablets of a sulfonamide for laryngitis.

Positive findings on physical examination were a sallow appearance; petechiae of the conjunctivae, mucous membranes of the nose, cheek, tongue, and palate; ecchymosis of the nail bed of the second finger, right; generalized lymphadenopathy; cardiac enlargement with a systolic apical murmur. Laboratory findings were: R.B.C. 2.3 M., hemoglobin 7 Gr., and W.B.C. 11,650, with a differential count of 86 per cent polymorphonuclear leucocytes, 11 per cent lymphocytes, and 3 per cent monocytes. Corrected blood sedimentation rate was 11 mm. (Wintrobe). Two platelet counts each showed less than 50,000 per cu. mm. The bleeding time was  $3\frac{1}{2}$  minutes, clotting time 4 minutes, and there was good clot retraction in 1 hour. The tourniquet test for capillary fragility was positive. The icteric index was 5, urobilinogen was 1:40, the urine showed a few red blood cells and leukocytes, the nonprotein nitrogen was 46 mg. per 100 cc., and the stools were guaiac negative. Electrocardiogram was interpreted as being within normal limits.

In the belief that the patient had rheumatic heart disease and subacute bacterial endocarditis the patient was treated with penicillin and given blood and plasma transfusions. On his fourth hospital day he had generalized convulsions for a few minutes, followed by confusion and disorientation. The seventh hospital day he had convulsions, became manic, lapsed into coma, and was incontinent. Episodes of convulsions continued intermittently and the patient remained comatose until his death on the eleventh hospital day, approximately 1 month after the onset of slight joint pains, and about 2 weeks after an acute episode following the administration of the sulfonamide drug. Showers of petechiae appeared throughout the last 4 days of his illness. Previously normal temperature, pulse and respirations became increased during his last 4 hospital days, the temperature varying during this period from  $101^{\circ}$  to  $104^{\circ}$  F., pulse averaging from 120-140, and respirations from 25-40 per minute. Six blood cultures showed no growth. A lumbar puncture taken after the patient's first convulsion was negative.

Autopsy revealed numerous petechiae and ecchymoses over the body and serosal surfaces. The heart weighed 400 Gr., contained many petechiae and ecchymoses over the visceral pericardium, but there was no evidence of valvular disease. The myocardium revealed focal areas of yellow and red discoloration. The lungs showed slight congestion and edema. The spleen weighed 200 Gr. and the liver 1300 Gr., and neither was remarkable. Slight generalized lymph node enlargement was present.

In view of the history, our previous experience with the disease, and the autopsy

findings, particularly the focal myocardial necrosis, a diagnosis of thrombocytic acroangi thrombosis was made. Histologic sections revealed the typical findings of platelet thrombosis in arterioles, capillaries, and venules, most prominent in the heart but also present to a considerable extent in the kidney, liver, brain, and lungs. Sternal marrow, again, showed lesions. Staining reactions were identical to those previously described. Additional findings were necrosis of vessel walls adjacent to thrombi and the presence of material in some perivascular areas apparently identical with that of the contiguous intravascular thrombus. Focal necrosis of the myocardium was prominent. There was considerable endothelial proliferation throughout the vascular lesions as well as platelet deposition, although the latter was the more marked. This case appears to emphasize again the possible role, at least in some cases, of a sensitivity factor in the pathogenesis of the disease.

While the proofs of this article were being corrected, one case of platelet thrombosis was reported by J. R. Carter, "Generalized capillary and arteriolar platelet thrombosis," *Am. J. M. Sc.* 213: 585-592, 1947. Another case was described by Engel, G. L., Scheinker, I. M., and Humphry, D. C., "Acute febrile anemia and thrombocytopenic purpura with vasothrombosis," *Ann. Int. Med.* 26: 919-933, 1947. In the latter case sensitivity to sulfonamide drugs was demonstrated clinically and splenectomy, performed when the patient was in coma, was followed by death on the second postoperative day.

We are indebted to the staff of the Fifth and Sixth Medical Services (Boston University), Boston City Hospital, for the clinical data of Cases 2 and 3.

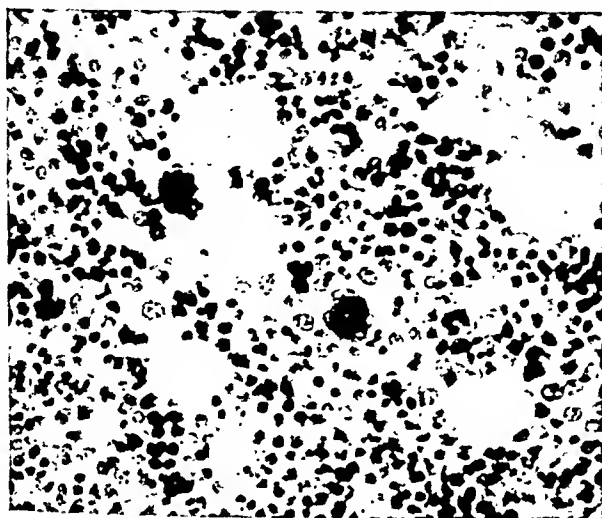
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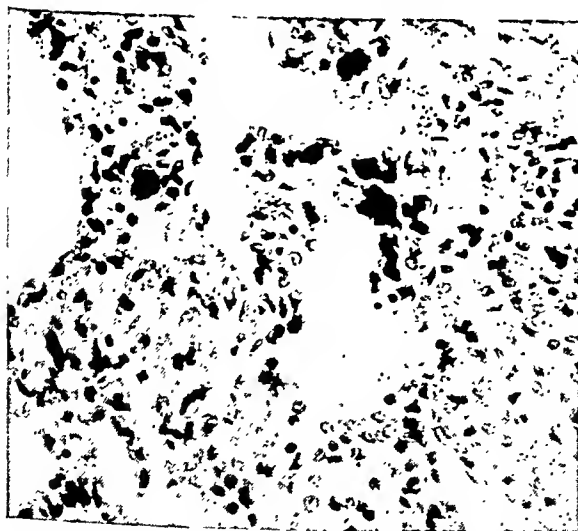
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hours. Two days later she was very restless, had severe frontal headaches and later was confused and disoriented. On the sixth day after admission, the patient lapsed into coma and died. A blood examination on the third day showed the following findings: Hgb 5.1 Gm. (33 per cent), R.B.C. 1.87 M., W.B.C. 9900, platelets 6000, hematocrit 17 per cent, color index 0.88, M.C.V. 91 cu. micra, reticulocytes 15 per cent. Differential count: polynuclear granulocytes: segmented 38 per cent, nonsegmented 6 per cent,



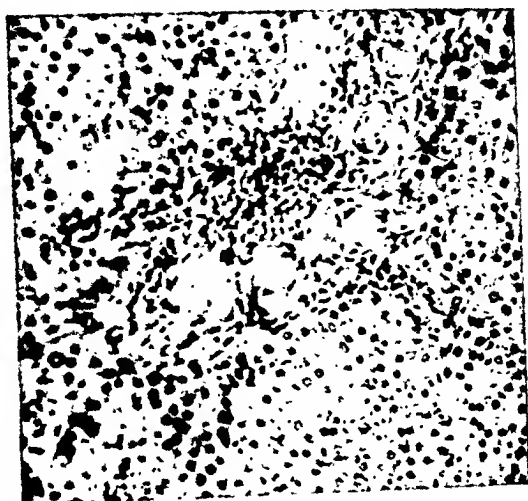
A. Bone marrow showing megakaryocytes (Magn. 340 X)



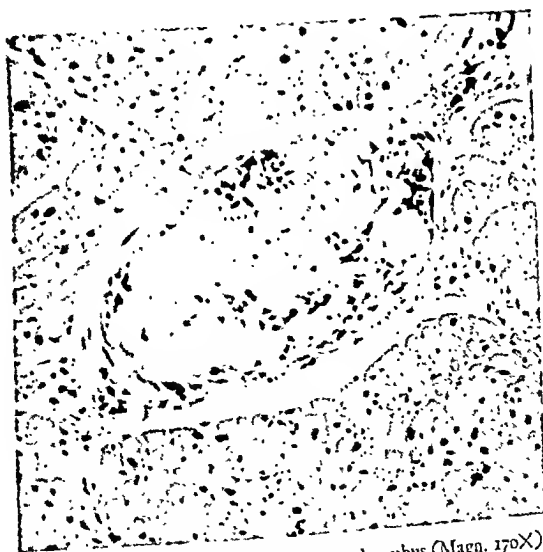
B. Lung with capillaries filled with megakaryocytes (Magn. 340 X)

lymphocytes 32 per cent, eosinophils 2 per cent, monocytes 12 per cent, neutrophil myelocytes 8 per cent, promyelocytes 1 per cent, myeloblasts 1 per cent, nucleated red cells 3/100 W.B.C. The sedimentation rate (Westergren) was 144 mm. Another blood smear taken 2 days later showed disappearance of the leukemoid reaction. Sternal puncture: marked erythroid hyperplasia; the erythroid-myeloid ratio was 2:1. The white cell series showed a preponderance of the more immature forms. Most of the megakaryo-

cytes, which were definitely increased in number, were normal in appearance. No signs of increased or decreased platelet production by these giant cells were demonstrable. The differential count of the bone marrow cells (3000 cells counted) was: megakaryocytes 1 per cent, histiocytes (reticulum cells) 1.5 per cent, proerythroblasts 0.3 per cent, basophilic erythroblasts 4.2 per cent, polychromatophilic erythro-



C. Liver showing thrombosed vessels (Magn. 170X)



D. Heart. Artery occluded by large thrombus (Magn. 170X)

blasts 31.2 per cent, orthochromatic erythroblasts 31 per cent, myeloblasts 3.6 per cent, promyelocytes 2.7 per cent, neutrophil myelocytes 11.5 per cent, metamyelocytes 0.6 per cent, band forms 1.8 per cent, polynuclears 3.6 per cent, eosinophilic polynuclears and myelocytes 4 per cent, lymphocytes 3 per cent. *Postmortem Report:* (Only the essential changes are given.) Gross findings: The skin was extremely pale and numerous areas of hemorrhages were present. The heart weighed 120 Gm. The epicardium

showed several petechial hemorrhages. The myocardium, especially that of the right ventricle, was mottled with pin-point and larger red spots. Small yellowish brown discolorations, measuring about 4-6 mm. in greatest dimension, were found in close association with the red areas. The liver weighed 850 Gm. The edges of the liver were rounded. The cut surface was a light yellowish brown and dry. The spleen weighed 110 Gm. It was quite soft. The cut section was dark red with faint trabecular outlines. The follicles were prominent. Both kidneys weighed 260 Gm. Their surface was mottled with a large number of tiny reddish dots, causing a flea-bitten appearance. Some of the mesenteric and periaortic lymph nodes were enlarged, soft and of a purplish red color. On sectioning, prominent follicles were noted. The brain disclosed two areas of subdural hemorrhage, one over the right postcentral gyrus and the other just beneath the tentorium, each measuring 2 by 2 cm. The right cerebral peduncle was the seat of an area of hemorrhage, measuring about 1 mm. in diameter.

*Microscopic findings:* Heart: Innumerable thrombi were seen throughout the sections. They were located in the capillaries, arterioles, and in smaller arteries. The thrombi consisted mainly of thrombocytes and some fibrin. Red blood corpuscles were not present in these thrombi. There were numerous foci of hemorrhages and early necrosis of heart muscle fibers located mainly in the neighborhood of the throm-



E. Blood vessel with platelet thrombus (Magn. 340X)

bosed vessels. In the papillary muscles the thrombi were especially numerous. Similar thrombi were seen in the lung, accompanied by small infarcts. Many capillaries were plugged with multinucleated cells resembling megakaryocytes. The liver also disclosed platelet thrombi. Here they were only found in the arterioles, while portal and central veins were free. A considerable amount of round cell infiltration was present in the periportal spaces. Platelet thrombi were also noted in the spleen, kidneys, urinary bladder, suprarenals, pancreas, and brain. The general architecture of the lymph nodes was well preserved. Numerous arterioles and capillaries were thrombosed. Patchy areas of hemorrhage and a distinct hyperplasia of the reticulo-endothelial cells were noted throughout the sections. Within the bone marrow there was marked increase of all cellular elements, particularly of the megakaryocytes. A few small thrombosed capillaries were also encountered.

#### DISCUSSION

In comparing the clinical and histologic features of our case with those of the 11 previously reported ones almost identical clinical and pathologic findings are encountered. From this it is quite obvious that we are dealing with a definite disease entity. However, most of the authors describing this disorder emphasize the his-

tologic pattern, but pay relatively little attention to the clinical aspects. In this study we wish to stress the clinical picture and its differential diagnosis. Only if the disease can be recognized ante mortem will an appropriate study of the pathogenic mechanisms become possible.

*Terminology:* In order to make a clinical diagnosis of this disease it is imperative that the diagnostician consider this rare disorder when confronted with a syndrome of thrombocytopenic purpura. A glance at the list of references<sup>1-9</sup> will demonstrate that no distinctive name has been attached to this disorder until now. The term "generalized arteriolar and capillary platelet thrombosis" is too long and cumbersome for practical use. We suggest the name "thrombotic thrombocytopenic purpura" for this syndrome, using the word "thrombotic" in the sense of "caused by thrombosis."<sup>10</sup> The name also emphasizes the most plausible theory of the pathogenic mechanism responsible for the thrombocytopenia, namely the disappearance of the platelets from the circulation due to the formation of myriads of platelet thrombi.<sup>2</sup>

*Race, Age and Sex.* The cases of "thrombotic purpura" reported thus far occurred in both whites and Negroes. The age of the patients varied from 9-66 years. Of the 12 reported cases (including our own) 3 were observed in children under 16. The disease seems to occur more frequently in females than in males, although the number of observations is too small for any definite conclusions. At present the ratio is 5:1 for the preponderance in females.

*Symptomatology.* The symptomatology of "thrombotic purpura" can be divided into 2 groups of manifestations. The first, which may be found in any of the thrombocytopenic purpuras, comprises petechiae and ecchymoses, epistaxis, melena, hematuria, and the laboratory findings of diminished number of platelets, prolonged bleeding time, positive tourniquet test, and poor clot retraction. The second group contains the features which may make the clinical diagnosis possible and will therefore be described in detail.

*Onset and Prodromal Symptoms.* "Thrombotic purpura" is an acute illness. Most patients give the history of an upper respiratory infection a few days or weeks prior to the appearance of the purpuric manifestations. Malaise, generalized aches and pains, arthralgia, throbbing headaches, and dizziness may be noted. There may be nausea and vomiting. Slight jaundice, the development of petechiae, and progressive pallor often bring the patient to the physician. At this time there is almost always fever of 100-102° F.

*Physical Examination* may now show the following abnormalities:

*Skin:* There are numerous painless petechiae and ecchymoses. Some observers have noted a peculiar brownish "café au lait" color.<sup>1,2</sup>

*Pallor:* Anemia is a constant and conspicuous feature. The severity of the anemia is out of proportion to any loss of blood which may have been observed. Sometimes the anemia may precede the external manifestations of the bleeding tendency due possibly to a hemolytic process.

*Jaundice and Hepato-Splenomegaly:* Manifest or latent icterus is a constant finding; the jaundice is always slight and of the acholuric or retention type. In one half

the cases the liver and spleen are palpable. This hepato-splenomegaly (always of moderate degree) may be of great importance for the differential diagnosis.

*Mental and Neurological Manifestations:* Almost all cases develop mental symptoms in the course of the disease. There is restlessness, confusion, irritability, incoherent screaming, muttering delirium, and stupor. These symptoms may be transitory, followed by lucid intervals. Convulsions are not infrequently seen. Besides these general symptoms of cerebral involvement, some cases show definite signs of focal lesions: facial weakness, hemiplegia, aphasia, dysphagia, apraxia, etc. Significantly, however, even these organic signs are often of a transient nature and thus differ fundamentally from the cerebral involvement (hemorrhage into the brain) seen in the other varieties of thrombocytopenic purpura in which this waxing and waning of signs is not observed. Rarely does the disorder begin with neurologic manifestations and is then followed by the other symptoms.<sup>4</sup>

*Fever:* Fever is almost always present. It is usually moderate in degree (100–102°F.) but terminal hyperpyrexia of 106–107°F. is not unusual. This elevation of temperature throughout the course of the disease is of diagnostic significance, as in "essential" thrombocytopenic purpura fever is usually lacking or slight.

*Cardiovascular System:* Acceleration of the pulse is noted in relation to the fever and anemia. Systolic murmurs over the base of the heart are common. Only in two cases was a "thrombotic nonbacterial endocarditis" observed.<sup>3,9</sup> The blood pressure is not altered by the disease.

*G.I. and G.U. tracts:* Vomiting and nausea are frequent. Melena is occasionally present. Hematuria is a constant finding but usually only discovered on microscopic examination. Smoky urine was seen in only 2 cases.<sup>2,9</sup>

*Laboratory Findings:* Bacteriological studies of the blood have not given any results. The urine almost always shows moderate albuminuria and micro-hematuria. There is no bilirubin present, but urobilinogen is markedly increased. Examination of the blood shows a varying degree of anemia of the normochromic normocytic variety. Hemoglobin values of 5 or even 3 grams are common. There is marked reticulocytosis but no spherocytosis, and the hypotonic saline fragility is unaltered. Nucleated red cells are a common finding in the peripheral blood, sometimes as many as 55 per 100 white cells being seen.<sup>2</sup> The serum bilirubin is moderately elevated. Studies of the hemolytic index<sup>11</sup> have not yet been performed. According to these criteria the anemia may well be classified as of the hemolytic type.

The white blood cells are at first moderately increased in number with a slight shift to the left. Later on a myeloid reaction may be seen (in half of the cases). The immature cells in the peripheral blood are myeloblasts, promyelocytes and myelocytes. This leukemoid picture is of great significance in the differential diagnosis; it may be only transient as in our cases, and may therefore be easily missed. When the disease is suspected, daily examination of blood smears may be a very valuable procedure.

Platelets are very much diminished or absent, the bleeding time is often prolonged, the tourniquet test positive, and clot retraction poor.

Only a few bone marrow studies by sternal puncture have been reported. They

usually show an erythroid hyperplasia and a normal or slightly increased number of megakaryocytes. In our case the number of megakaryocytes was definitely increased. The giant cell pattern seemed to be a normal one; unfortunately no quantitative studies using the method of Limarzi and Schleicher were performed.<sup>12</sup> Table 1 lists the essential findings in the 12 observations available.

In summary then the clinical picture of "thrombotic thrombocytopenic purpura" is that of an acute febrile illness characterized by a severe anemia out of proportion to any observed blood loss, mild acholuric jaundice, hepato-splenomegaly in half of the reported cases, bizarre and intermittent mental and neurological symptoms and signs of a transient nature and a leukemoid reaction in the peripheral blood, besides the hemorrhagic manifestations commonly seen in thrombopenic purpura.

#### DIFFERENTIAL DIAGNOSIS

With the clinical picture of purpura, determination of the platelet count will readily demonstrate whether the presenting syndrome belongs to the thrombocytopenic or nonthrombocytopenic variety. If a diminution of platelets establishes the diagnosis of the former, the possibility of "thrombotic purpura" should be considered.

An entirely satisfactory and generally accepted classification of the various thrombocytopenic syndromes is not available at the present time because of our very incomplete knowledge of the pathogenic mechanisms involved. If one accepts the theory that the megakaryocytes of the bone marrow produce the platelets<sup>12,15</sup> one must also accept the concept that their production and release are likewise controlled by various metabolites or hormones. The existence of a splenic inhibitor of the marrow has recently been demonstrated.<sup>14-21</sup> The role of such a "splenic hormone" has, however, not yet been sufficiently studied in the various types of purpuras. Although it may be premature to base any classification of the thrombocytopenic syndromes on our present knowledge of functional principles, such a correlation is attempted in this paper. The following types of thrombocytopenic purpura may be distinguished:

1. *Diminution of platelet production caused by aplasia or hypoplasia of the megakaryocytic apparatus.* This is seen in the aplastic anemias of the "idiopathic" or of the symptomatic type (osteosclerotic anemia, effect of benzol or gold salts, x-rays, radium, etc.). In these conditions there is anemia, leukopenia, and thrombocytopenia, the marrow is empty, and the course of the disease is usually a chronic one. Histologically the few remaining megakaryocytes appear to be normal.<sup>15</sup>

2. *Diminution of platelet production due to interference by "foreign" cells.* To this group belong the diseases in which one can demonstrate an "invasion" of the bone marrow by foreign cells. (Gaucher cells, cancer or sarcoma metastases, myeloma cells, etc.). The disorders which show a proliferation of the immature white cells, i.e. the acute and chronic leukemias, also fall into this group. Severe hyperplasia of the erythroid apparatus is sometimes also accompanied by thrombocytopenic purpura, as in severe pernicious anemia or in primary hypochromic anemia. There may be either mechanical interference or a metabolic disturbance. The antiperni-

TABLE 1.—Clinical Manifestations in Cases Observed

No. of Case	Name of Author	Race	Age	Sex	Prodromal Symptoms	Hemorrhagic (Petechiae, Ecchymoses)	Pallor	Jaundice	Hepatosplenomegaly	Splenomegaly	Mental and Neurological Manifestations	Fever 101-104°F.	G.I. Symptoms	Hematuria	Severe Anemia	Faekel-mold Reaction	Thrombocytopenia
1	Moscowitz	White	16	F	weakness, arthralgia	present	present	absent	absent	absent	paralysis of left facial, paralysis, coma not reported	present	not reported	?	present	not reported	not reported
2		White	9	F	listless, pale, headache	present	present	present	absent	present		present	not reported	gross hematuria	present	not observed	present
3	Baehr, Klemperer, and Schiffrin	White	18	F	brownish pallor, weakness, vertigo, headache	present	present	present	absent	absent	clonic twitching, vertigo, headache, irrationality	present 107° terminal	not reported	present	present	present	present
4		White	22	F	not reported	present	present	absent	absent	absent	intermittent delirium, restlessness, stupor	present	nausea, vomiting, melena	present	present	present	present
5		White	48	F	arthralgia, upper respiratory infection	present	present	present	absent	absent	terminal right hemiplegia, coma	present	not reported	present	present	present	present
6	Gitlow & Goldmark	White	18	F	upper respiratory infection, malaise, vomiting	present	present	absent	present	absent	right hemiplegia	present	vomiting	present	present	not reported	present
7	Altschule	White	50	F	general malaise, abdominal pains, fatigue, arthralgia	present	present	present	present	present	headaches, dizziness, confusion, restlessness, delirium	present 107°	vomiting, melena	present	present	not observed	present
8	Bernheim	White	33	F	weakness, throbbing headaches	present	present	absent	absent	absent	convulsions, coma	present	anorexia	present	present	present	present
9	Trobaugh et al.	White	24	M	upper respiratory infection, malaise	present	present	present	absent	absent	restless, uncooperative	present	vomiting	present	present	bilast?	present
10	Carter	Negro	66	M	aplasia, begin with neurologic changes	no petechiae or ecchymoses but thrombopenia	present	present	not reported	not reported	aphasia, apraxia, dysphagia, spasticity	present	not observed	present	present	not observed	present
11	Engel, Selekner & Humphrey	Negro	15	F	upper respiratory infection, vomiting, headache	present	present	present	present	present	delirium, disorientation, spastic paresis	present	vomiting	gross hematuria	present	present	present
12	Own case	White	11	F	weakness, upper respiratory infection	present	present	absent	present	present	headache, disorientation, stupor	present 106.1° terminal	nausea	present	present	present	present

cious principle appears to have a definite effect on the megakaryocytes. Administration of liver produces an increase in platelets in pernicious anemia which precedes the elevation of the reticulocyte count in the peripheral blood.<sup>22</sup> In all these disorders the diagnosis can usually be made without great difficulty from the bone marrow smear. Histologically the megakaryocytes appear to be normal.<sup>15</sup>

Severe hemolytic anemia of the acquired type with thrombocytopenia is unusual. In nocturnal hemoglobinuria (Marchiafava-Micheli syndrome) diminution of platelets is frequently seen but not accompanied by purpuric manifestations.<sup>23</sup> The chronic character of the disease, the hemoglobinuria, and the nocturnal rhythm are sufficiently distinct features.

3. *Inhibition of release of the platelets.* To this group belong the cases of thrombocytopenic purpura which have been classified as "Werlhof's disease" or "essential purpura." Recent investigations have demonstrated that this syndrome is caused by an inhibitory influence of a "splenic hormone" on the bone marrow.<sup>14-21</sup> The number of megakaryocytes is increased but they show great diminution in platelet production;<sup>15</sup> following splenectomy the production of platelets from the megakaryocytes often becomes demonstrable to an extreme degree.<sup>15</sup> This "splenic inhibition" syndrome may be present in an acute or chronic form. In the acute type, which is of special interest in the differential diagnosis of "thrombotic purpura," fever is absent or slight, and when present is caused by secondary infection.<sup>24</sup> Anemia is present only in proportion to the blood loss. Neurologic manifestations occur but are caused by gross hemorrhage into the brain and are not of an intermittent type. Hepatomegaly is not seen and splenomegaly is a rare finding. Leukemoid reactions are rarely encountered.

Splenic inhibition may also occur symptomatically in liver cirrhosis, in congestive splenomegaly and in Felty's syndrome, but the clinical picture in these latter disorders is usually of such a nature that it does not resemble "thrombotic purpura." The megakaryocyte pattern in the symptomatic splenic inhibition may be the same as in Werlhof's disease<sup>25</sup> or may be a normal one.<sup>15</sup> Further extensive marrow studies in these disorders are indicated.

4. *Allergic purpura.* The fourth group comprises the cases of allergic thrombocytopenic purpura which may be caused by allergy to drugs (sedormid, quinine, sulfa compounds, etc.), to food stuffs,<sup>26</sup> or to bacteria or viruses. The point of attack of the pathogenic mechanism operating in this group is not clear. Schwartz<sup>27</sup> has recently pointed out that in such cases there is a considerable increase of the eosinophils in the marrow even in the absence of eosinophilia in the peripheral blood. No such increase has been observed in "thrombotic purpura." Furthermore, the course of the allergic purpuras is never as stormy or rapid as in the thrombotic variety.

5. *Infectious or toxic thrombocytopenic purpura.* This type is seen occasionally but by no means regularly in infectious diseases as subacute bacterial endocarditis, typhoid fever, smallpox, infectious mononucleosis and lupus erythematosus.<sup>15</sup> To this group also belongs Minor's thrombocytopenic purpura with lymphocytosis.<sup>23</sup> The mechanisms causing these purpuras are unknown. S. O. Schwartz and one of us (K. S.) have recently observed a case of infectious mononucleosis with thrombo-

cytopenia in which the marrow showed a marked eosinophilia. It is therefore conceivable that an allergic mechanism may also be in operation in some of these infectious purpuras.

"*Thrombotic purpura*" can easily be differentiated from the first and second group. If a leukemoid picture is present, exclusion of leukemia is necessary by means of marrow studies. It can also be ruled out on clinical grounds. Thrombocytopenia is regularly found in acute leukemia which, however, shows a different blood picture (hiatus leukemicus, Nageli). In chronic myelogenous leukemia, purpura is only seen in the last stages of the disease. Allergic purpura can also be differentiated by means of the stormy, febrile course of "*thrombotic purpura*" and the absence of eosinophilia in the marrow. Splenogenic "essential" purpura rarely shows the degree of anemia, fever, and the transitory neurologic and hematologic responses seen in "*thrombotic purpura*." The greatest differential diagnostic difficulties may be encountered in the symptomatic purpuras of the infectious or toxic type; often, however, the underlying disorder may manifest itself so obviously that the diagnosis can be established. In subacute bacterial endocarditis, for example, the blood culture, the history of a pre-existing heart disease and the type of murmurs may be of great help in the differentiation. A lymph node biopsy may also be of great diagnostic value if the characteristic histologic pattern can be found. Although the recognition of "*thrombotic purpura*" is not simple, we are certain that when familiarity with this clinical picture increases, ante mortem diagnoses will certainly be made more often in the future.

#### PATHOLOGY AND PATHOGENESIS

The remarkable histologic pattern which is found at autopsy in the cases of "*thrombotic purpura*" consists of innumerable thrombotic lesions within the capillaries and the small arterioles. There is general agreement that these thrombi are composed of masses of platelets. Only a small amount of fibrin and no erythrocytes are found in the thrombi. In the capillaries of the lungs characteristic megakaryocyte thrombi may be present. This is a frequent but inconstant finding. The endothelial lining of the vessels shows proliferation of varying degree. Most observers believe that this endothelial reaction follows the formation of the thrombi and is therefore a secondary phenomenon. Altschule<sup>5</sup> considered the possibility of a primary vascular disease with secondary thrombus formation. In support of the first mentioned interpretations are the observations that no evidence of endothelial damage is demonstrable in any other parts of the vascular tree where no thrombi are found, but that thrombosis may be present without any noticeable proliferation of the endothelium. In the case of Carter,<sup>8</sup> however, necrosis of the capillary wall with extrusion of the thrombotic material into the adjacent tissue was seen, and Trobaugh and al.<sup>7</sup> found swelling of the capillaries without thrombi. From the histologic findings it is quite obvious that the formations of the platelet thrombi take place in a succession of attacks.<sup>5</sup> One is tempted to speculate whether the transitory character of some of the manifestations of "*thrombotic purpura*" may not be related to such paroxysms of vascular occlusion.

There is also general agreement amongst all pathologists who have studied

"thrombotic purpura" that this disorder is in no way related to generalized disseminated lupus or polyarteritis. The vascular lesions in these latter diseases consist in primary alterations of the wall of the blood vessels, whereas in "thrombotic purpura" the platelet thrombi are the outstanding feature of the histologic pattern.

Baehr, Klemperer and Schiffrin<sup>2</sup> suggested that the myriads of platelets caught in the thrombi may account for the lack of thrombocytes in the circulating blood. The increase of the number of megakaryocytes in the bone marrow could then be explained as a compensatory reaction. This "exhaustion theory" may plausibly account for the diminution of platelets in the blood but does not satisfactorily explain the bleeding tendency. Bedson's<sup>29</sup> repeatedly confirmed experiments<sup>30</sup> have shown that lack of platelets produced by intravenous injection of agar or anti-platelet serum does not result in purpura; however, if the capillary endothelium is damaged by means of an anti-red cell serum, hemorrhage occurs. It must therefore be assumed that such a damaging "toxic" factor is also operative in "thrombotic purpura."

No explanation is available for the constant presence of a very severe anemia. It is unlikely that the severe anemia is due to "hemorrhage into the tissues." The hemosiderosis of the tissues is only slight in almost all observed cases and it must therefore be inferred that the anemia is caused by intravascular destruction of the red cells.

The etiology of the disease is unknown. There is no evidence either for or against the assumption that "thrombotic purpura" is caused by some unknown type of infection. The presence of a powerful toxin could explain the hemolytic anemia, the capillary damage, and the change in the clotting mechanism which are present in this disorder. That some abnormality of the clotting mechanism is present seems very likely but no systematic studies have been performed until now. Bernheim<sup>5</sup> examined cadaver blood for platelet agglutinins but was unable to demonstrate such antibodies.

Schwartzman, Klemperer and Gerber<sup>31</sup> produced lesions similar to those seen in "thrombotic purpura" experimentally in animals by inducing the Schwartzman phenomenon. These findings may offer some kind of explanation of this enigmatic disease.

#### PROGNOSIS AND TREATMENT: FURTHER INVESTIGATIONS

Our knowledge of "thrombotic thrombocytopenic purpura" is based on cases with a fatal outcome. Whether milder, unrecognized variations of the disease exist, from which recovery is possible, is unknown. One case showed transitory improvement.<sup>9</sup> Splenectomy was performed in two cases but the patients died immediately after the operation.<sup>2,9</sup> On theoretical grounds there seems to be no indication for such an operation, as no inhibition of the marrow appears to be present. If the disease is diagnosed *in vivo*, treatment with heparin or dicoumarol may be indicated.

If in the future an ante mortem diagnosis of "thrombotic purpura" should be made, the following studies might be of interest.

It has been pointed out that the anemia present is of the hemolytic type; the

mechanisms involved are unknown. It may be worthwhile to look for incomplete immune bodies attached to the red cells by means of an anti-human globulin serum; this procedure has proved its value in cases of acquired hemolytic anemia.<sup>32</sup> Differential fragility tests<sup>33</sup> using lysolecithin and saponin as hemolytic agents may also be of interest.

Liver function tests (thymol turbidity, bromsulphthalein) should be performed in order to determine the role which the liver plays in the pathogenesis of the icterus. It is well known that inability to remove bilirubin from the blood is one of the first signs of hepatic damage.

Assays of splenic "hormone" may be of interest. Studies of the clotting mechanisms step by step are definitely indicated. Particular attention should be given to the newly discovered plasma factors of Owren.<sup>34</sup> Quantitative evaluations of the number and type of the megakaryocytes using the technique of Limarzi and Schleicher<sup>12</sup> should also prove valuable. Bacteriologic studies, particularly inoculation of animals, should be attempted.

#### SUMMARY

1. "Thrombotic thrombocytopenic purpura" is the name which we propose for a rare but well-defined disorder which manifests itself clinically as an acute febrile illness and which is characterized by (a) petechiae and ecchymoses, thrombocytopenia, prolonged bleeding time and poor clot retraction, (b) by a severe anemia out of proportion to any observed blood loss, (c) by mild acholuric jaundice, hepatosplenomegaly, (d) by bizarre and intermittent mental and neurologic symptoms and signs, and (e) by a transient leukemoid reaction in the peripheral blood.

2. This clinical picture must be correlated with a remarkable histologic pattern, namely the presence of myriads of platelet thrombi in the small arterioles and capillaries of almost all organs of the body.

3. Eleven such cases have been described in the literature. One case of our own is added.

4. The clinical features of this disease are detailed and the differential diagnosis is discussed. It is emphasized that if the physician is familiar with this syndrome a correct clinical diagnosis may become readily possible.

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# CHEMOTHERAPY OF MULTIPLE MYELOMA; THE USE OF ANTIMONY

(PRELIMINARY REPORT)\*

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**M**ULTIPLE myeloma is a neoplastic, infiltrative disease of the bone marrow. The histological origin of the myeloma cells, whether of lymphatic, myeloid or other origin, is still a matter of discussion. Nor is the position of multiple myeloma among other neoplastic diseases, in particular its relationship to leukemia, sufficiently clarified. It shares with the other neoplastic diseases both the mystery of its origin and the lack of any effective treatment.

However, there is one characteristic which distinguishes myeloma from other neoplastic diseases, namely the peculiar abnormalities in its protein metabolism. The main biochemical features of multiple myeloma are the Bence-Jones proteinuria, discovered as early as 1845 only a few years after the first case of multiple myeloma had been described, and the hyperproteinemia found a half century later and finally identified as being essentially due to hyperglobulinemia. This hyperglobulinemia is the basis for many laboratory tests characteristic of multiple myeloma, such as rouleaux formation, rapid sedimentation rate of red cells, formol gel test, etc.<sup>1</sup>

In addition to multiple myeloma, there are at least 3 other diseases likewise characterized by the presence of hyperproteinemia and hyperglobulinemia, but unlike multiple myeloma these diseases are of well established etiology. They are kala-azar, lymphogranuloma venereum, and schistosomiasis. These diseases have varied etiologic agents: protozoan Leishman-Donovan bodies in the first instance, a filtrable virus in the second, and metazoan helminthes in the third.

Despite the wide range of etiologic agents in these diseases, they have two common denominators: (1) the frequent occurrence of hyperglobulinemia, and (2) the favorable response to the same therapeutic agent, namely to antimony compounds.<sup>2</sup>

The therapeutic action of antimony has not been clearly established. However, since its activity is largely confined to the above mentioned diseases, notwithstanding their various etiological agents, we may assume that its effects are linked in some way with the biochemical characteristics which the 3 diseases have in common, that is, the hyperglobulinemia.

Based on the assumption that antimony acts on diseases with hyperglobulinemia, it was a natural step to assume that the drug might be found therapeutically effective in another disease with hyperglobulinemia, namely in multiple myeloma.

The experimental treatment of multiple myeloma with antimony was begun

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in the spring of 1943 and has been continued with some interruption to the present. Early in 1945 Snapper began to treat patients with multiple myeloma with stilbamidine, another drug recently shown to be effective in kala-azar, also on the basis of the occurrence of hyperglobulinemia in both diseases.<sup>3</sup>

The dosage and the preparations used by us have been repeatedly changed during the work. At first we used preparations of tartar emetic which, because of its toxicity, could be administered only in small doses; later less toxic antimony compounds were used and at present neostibosan is being given. A full course of treatment consists in the injection intravenously of 15 Gm. of the latter preparation in divided doses of 0.3 Gm. given at suitable intervals. Special precautions, including frequent urine and blood examinations, are necessary in cases showing renal involvement.

This paper is a preliminary report based on our experience with this method of treatment on 7 patients with multiple myeloma. In evaluating the influence of antimony on multiple myeloma it is necessary to realize the possibility of occasional spontaneous remissions in this disease, as well as of a prolonged course over a period of years with a relative freedom from symptoms, and the occasional sensitivity to radiation with amelioration of pain in some cases.

This occurred in one of the first patients (case no. 2) treated with antimony, who, in the period before treatment showed a very prolonged course with frequent symptomatic remissions including spontaneous healing of pathological fractures. At the time treatment was instituted, multiple painful external tumors were present over the clavicle, ribs, and arms. The first course of antimony thioglycollamide was followed by a course of x-ray therapy, since the latter had relieved pain on previous occasions. The visible tumor masses regressed promptly following this combined treatment. However, similar lesions soon appeared at different sites of the skeleton, some of them only to disappear again following another course of x-ray therapy; the pain was also greatly relieved.

The remarkable radiosensitivity of the tumor masses seen in this particular patient following a course of antimony treatment could not be attributed with certainty to the drug. This case, already briefly reported,<sup>4</sup> will be the subject of a separate publication in which the difficulties in evaluating treatment of multiple myeloma will be discussed at length.

The results obtained in another patient (case no. 4) were more conclusive. Large external masses over the anterior and posterior surface of ribs were present together with numerous punched out areas in the bones. The visible tumor masses had been noticed by the patient about 2 years prior to admission, and were progressively increasing in size. The patient received six x-ray treatments, but without relief of pain or reduction in the size of the tumor masses. On admission to the hospital in 1945, a first full course of neostibosan treatment, followed by x-ray therapy applied to the site of the tumor masses, was given. At the completion of neostibosan injections, and before further x-ray treatment, the visible tumors were found to be decreased in size, and they completely disappeared soon after the subsequent x-ray therapy. To date, 8 months after this treatment, no external tumor masses have reappeared.

Examinations of the bone marrow were repeated at frequent intervals, both during and after the course of treatment; both sternal and iliac bone aspirations were studied. We are aware of the fact that numerical variations of the plasma cell content of bone marrow aspiration may occur on repeated examinations, even when simultaneously performed at different sites. It is nevertheless interesting to

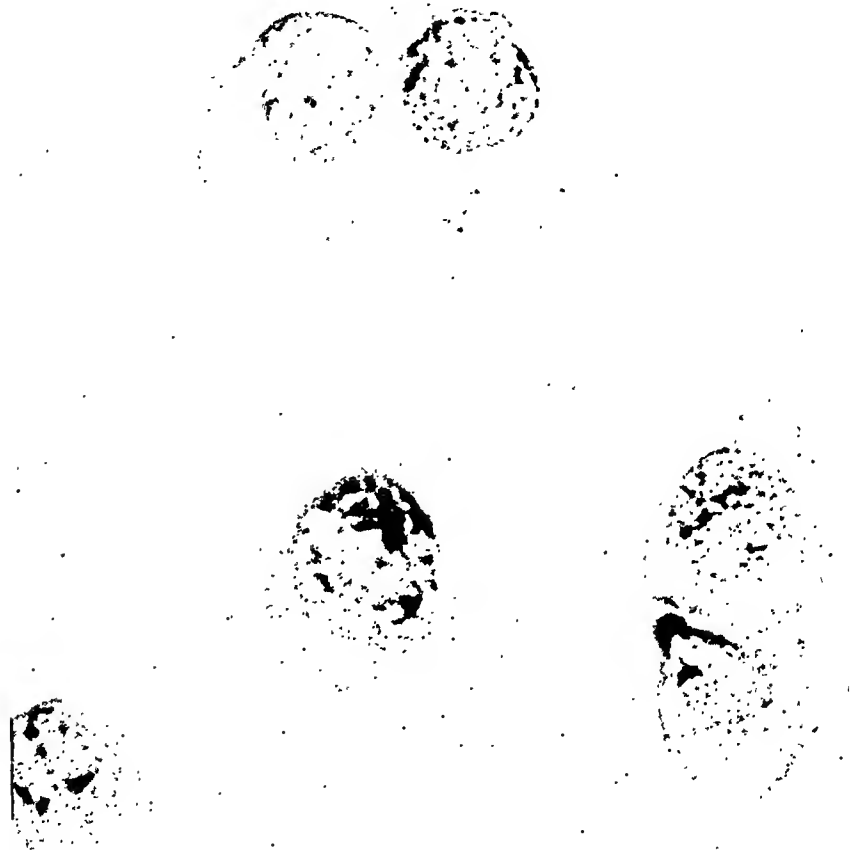


FIG. 1. Case 4. Plasma cells in the bone marrow after ten injections of 0.3 Gm. neostibosan. Occasional granules are seen in the plasma cells.

note that following antimony treatment the percentage of myeloma cells in the differential count was frequently found to be diminished, and the number of normal myeloid and erythroid elements increased. Another index of the degree of myelomatous infiltration of the bone marrow would be the degree of anemia which is almost certainly due to displacement of the hematopoietic tissue by the myeloma cells. The observations concerning the influence of antimony treatment on the anemia of multiple myeloma are now being conducted.

Changes in the morphologic appearance of the plasma cells were more significant than the quantitative variation. The appearance of metachromatic, basophilic granulation in the cytoplasm of myeloma cells in 2 of the 7 cases treated with antimony, constituted the most conspicuous change. These granules were seen in greater numbers only after repeated courses of antimony injections, and were usually lacking before completion of the full course of treatment. The photomicrographs shown in figures 1 and 2 reproduce the granulated myeloma cells in a

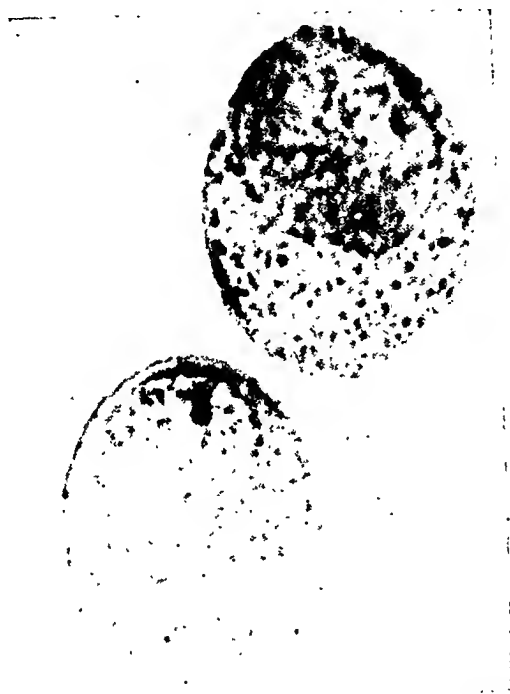


FIG. 2. Case 4. Plasma cells in the bone marrow after two courses of neostibosan injections. Plasma cells containing numerous granules.

patient receiving antimony (case no. 4). It is noteworthy that no other bone marrow elements were affected in this way by the antimony treatment.

In some cases, the plasma cells, following a course of antimony, assumed an appearance resembling that of a medium size lymphocyte.

No definite changes were observed in the hyperglobulinemia in patients receiving antimony.

The case (no. 6) of M.K., a 53 year old woman will be cited as an example of a striking relief of pain following combined antimony and radiotherapy. This patient's chief complaint was very severe pain in the back for the past year. The diagnosis of multiple myeloma was made on the basis of bone marrow findings and x-ray studies of the bones; there was also a moderate hyperglobulinemia,

but no Bence-Jones proteinuria. The patient was given (October 11, 1946 through October 26, 1946) a full course of neostibosan, followed by x-ray treatment. Progressive relief of pain was seen in the course of this treatment. This patient, who was bedridden and unable to turn in bed, is now being fitted for a brace which will enable her to get out of bed; the analgesics which the patient was receiving are being withdrawn.

In general, our observations on antimony are strikingly similar to those of Snapper, using stilbamidine, a drug containing no antimony.

Brief summaries of the case reports of the 7 patients treated with antimony follow:

#### CASE NO. 1

Patient R. S. (Hospital admission no. 362573) was a 58 year old woman, first seen on August 6, 1943. She complained of low back pain of 2 months' duration. Physical examination revealed tenderness over the spinous processes of L<sub>4</sub> and L<sub>5</sub> and the upper sacrum. The blood count showed hemoglobin 31 per cent, red blood cells 1,900,000 and white blood cells 6,100 per cu. mm. The differential count was normal except for an occasional atypical plasma cell which contained one or more nucleoli. The possibility of plasma cell multiple myeloma was at once suggested by the appearance of these cells. Sternal marrow aspiration confirmed the diagnosis of myeloma, revealing 56 per cent plasma myeloma cells.

Other blood studies were: urea nitrogen 64 mg. per 100 cc., phosphorus 5 mg. per cent, calcium 10.4 mg. per cent, total protein 7.3 Gm. per cent, globulin 4.4 Gm. per cent, alkaline phosphatase 4 King-Armstrong units, sedimentation rate 18 mm. in 8 minutes. The urea clearance was 11 per cent. The blood serum showed an immediate positive formol gel reaction. Using the ordinary heat test at pH 5 the urine was found to contain large quantities of Bence-Jones proteoses and to be consistently free of albumin. There were occasional hyalin and granular casts. Urinary concentration tests showed a specific gravity of 1.012 to 1.015. Roentgenographic examination of the skull showed numerous circular punched out lesions throughout the bones of the calvarium. There were numerous areas of bone destruction of the lower ribs, lumbar vertebrae, and pelvis and a pathological fracture of the body of the first sacral vertebra.

On August 20, 1943 and on the two following days the patient received intravenous injections of 10 cc. of 1 per cent tartar emetic. The injections had to be stopped because of a shocklike reaction following the third injection. The patient stated that during the few weeks following the injections, she was able to move more freely and perform some housework. However, in the last week of September she suddenly developed signs of paresis of the low extremities and severe backache. She was admitted to Mount Sinai Hospital where collapse of several lumbar vertebrae was found with evidence of spinal compression. The course was progressively downhill and she died on October 1, 1943. A postmortem examination was not obtained.

#### CASE NO. 2

Patient A. W. was a 17 year old boy (Hospital admission no. 105534) with a history of pain in the left hip for 3 years. In June 1942 he sustained a pathological fracture of the left hip. The diagnosis of multiple myeloma was made on the basis of bone marrow studies which showed the presence of 28 per cent plasma cells. The diagnosis was substantiated by the presence of multiple punched out areas in x-ray films of the skull and ribs and long bones. Urine examinations showed the presence of Bence-Jones protein. The blood proteins were normal, as well as blood phosphorus, calcium and phosphatase.

Biopsy of a lesion in the skull, localized in the x-ray, was performed on March 8, 1943. The pathological report was plasma cell myeloma.

In 1944 there were noted, in addition to the bone lesions seen in the x-ray films, multiple tumor masses over the clavicle and the ribs; also on the anterior surface of the ribs and over the sternum. The lesions ranged from pea size to the size of a large fist. It was noted that some of these lesions regressed spontaneously.

Patient received intensive radiotherapy to the various affected areas.

The first course of x-ray treatment at Montefiore Hospital was given to the lesion in the left tibia. He received 6250 R to that area given through 3 different portals. Subsequently, he received similar treatments to the various affected parts including the skull, right hip, right upper arm, right forehead, right mastoid area, right clavicle, left clavicle, left knee, etc. Marked relief of pain was observed, although x-ray studies showed definite progression of the lesions. He also received radiotherapy to the left shoulder, chest, lower back, lower abdomen, and right thigh, all with considerable relief of pain but with no change in the appearance of the lesions on x-ray.

Treatment with antimony was started on April 25, 1944. Only two intravenous injections of 5 cc. of 1 per cent tartar emetic were given. These were discontinued because of a shocklike reaction. Antimony was resumed on September 15, 1944 with daily intravenous injections of 20 cc. of 5 per cent antimony thioglycollamide continued through October 9, 1944. This course of treatment was followed by another course of radiotherapy. A rapid regression of a huge mass over the sternum and of the smaller tumors over the clavicles was noted after this combined treatment. However, in view of the spontaneous regression of some other visible tumors, previously observed in this patient, the value of the treatment was difficult to appraise.

In the fall of 1944, the patient developed signs of uremia, spinal compression with appearance of a neurogenic bladder, and pyelonephritis developed. The patient died in December 1945 of uremia. Autopsy showed multiple myeloma, with myeloma kidney.

#### CASE NO. 3

Patient A. H. (Hospital admission no. 39350), a 64 year old male, was admitted to the hospital in November 1944 for pain in the back and right leg of two months duration. Physical examination was negative except for percussion tenderness over the lumbar spine and over the right thigh. The diagnosis of multiple myeloma was made on the basis of sternal marrow aspiration which revealed 51 per cent plasma myeloma cells in differential count. X-rays of the bones showed extensive destruction of the L1 and L2 vertebrae and marked degree of general decalcification of the skeleton. Blood studies showed: total serum protein 12.4 Gm. per cent, albumin 3.4 Gm. per cent, globulin 9.0 Gm. per cent, calcium 10.2 mg. per cent, phosphorus 4.3 mg. per cent, alkaline phosphatase 0.2 Bodansky units, hemoglobin 10.5 Gt. per 100 cu. cm., red blood count 3,500,000 per 1 cu. mm., white blood count 5,500 with a normal differential. Urine examination showed traces of albumin but was negative for Bence-Jones protein.

Patient was given 4 injections of 5 cc. of 1 per cent tartar emetic intravenously (May 30, May 31, June 2, June 3, and June 4, 1945). Because of shocklike reaction which followed, these were discontinued. Radiotherapy was then given over lumbo-sacral area and over the right mid-thigh. In July marked relief of pain was noted. The serum protein at that time showed: total protein 7.0 Gm. per cent, albumin 2.9 Gm. per cent, globulin 2.1 Gm. per cent, alkaline phosphatase 3.7 Bodansky units. However, in October 1945 the patient developed pneumococcal pneumonia of the entire left lung to which he succumbed in spite of intensive penicillin treatment. Autopsy revealed plasma cell myeloma involving bone, spleen and lymph nodes.

#### CASE NO. 4

Patient D. C. (Hospital admission no. 110504), a 47 year old male, was admitted in April 1946 with a history of pain in the lower left chest for the last 2 years and pain in both hips for the last year with a loss of 25 pounds during this time; also progressive general weakness.

About 2 years ago the patient noted the appearance of two masses in the outer part of the left chest and in the left axillary region which have been progressively increasing in size in spite of x-ray treatments given about a half year ago. On admission the mass in the left axilla measured 10.5 cm. length, 8 cm. wide and 2 cm. deep. The mass over the left chest measured 7 cm. x 6.5 cm. x 3 cm. Blood count showed: hemoglobin 60 per cent, red blood count 3,150,000, white blood count 9,500, normal differential. Blood chemistry determinations: total serum protein 11.8 Gm. per cent, albumin 3.1 Gm. per cent, globulin 8.7 Gm. per cent, calcium 10.6 mg. per cent, phosphorus 3.8 mg. per cent, alkaline phosphatase 6 Bodansky units, urea nitrogen 8.9 mg. per cent. The urine was negative for Bence-Jones protein, sugar and albumin. X-ray studies showed multiple areas of destruction in the frontal and parietal bones of the skull, and bone destruction in the eighth and tenth rib and pathological fracture of ribs in the axillary region. Shadows

of the 2 above mentioned visible masses were seen. There was also compression of the fifth lumbar vertebra. Sternal marrow aspiration showed 38 per cent plasma myeloma cells.

On April 5, 1946 the patient was started on a course of neostibosan treatment consisting of a daily intravenous injection of 0.3 Gm., until the total amount of 15 Gm. was reached. On May 17, on completion of the first course of treatment the visible tumors had decreased in size measuring: 4.5 cm. x 4.0 cm. x 0.5 cm. over the left posterior thorax, and 6.5 cm. x 8.0 cm. x 3.0 cm. in the left axilla. A course of neostibosan treatment was repeated twice again, in May and August 1946. The last treatment was followed by a course of radiotherapy. At the end of August 1946, following this combined neostibosan and x-ray treatment both visible tumor masses had completely disappeared. At that time the patient stated that there was improvement of his general condition and considerable relief of pain. Urine had been negative for Bence-Jones protein throughout the patient's stay in the hospital. Blood urea nitrogen after treatment on May 17 was 10 mg. per cent. The serum protein showed some decrease.

TABLE 1.—*Serum Protein in Case 4*

Date	Total Protein	Albumin	Globulin
	Gm.	Gm.	Gm.
May 17, 1946.....	9.9	3.1	6.8
July 3.....	8.3	2.3	5.8
Aug. 14.....	10.6	3.7	6.9
Oct. 23.....	7.1	2.1	4.9
Jan. 20, 1947.....	7.3	2.0	5.3

Bone marrow studies repeated on August 14, 1946 and October 26, 1946 showed a diminished number of plasma cells (16 per cent and 23 per cent respectively). Basophilic granulation was seen in the cytoplasm of the plasma cells.

In September 1946 the patient was allowed to walk but sustained pathological fracture of the hip and was immobilized in bed with application of traction. This patient is still in the hospital under observation.

## CASE NO. 5

Patient M. W. (Hospital admission no. 110282), a 54 year old colored woman, was first seen on June 15, 1946 with a history of numbness in the hands for 2 years followed by pain in the back, elbows, knees and wrists. X-ray examination of the bones showed areas of bone destruction in both scapulae and in several of the ribs, clavicles, humeri and both radii. The urine test for Bence-Jones protein was positive. Blood chemical determinations on admission showed: total protein 7.0 Gm. per cent, albumin 5.0 Gm. per cent, globulin 2.0 Gm. per cent. The hemoglobin was 10 Gm. per 100 cc., red blood count 3,120,000 per cu. mm. and white blood count 6,000 per cu. mm., with normal differential. Bone marrow studies were characteristic of multiple myeloma, showing 20 per cent plasma cells.

Patient was given a course of neostibosan from June 14 through July 7. On August 30, 1946 another course of neostibosan treatment was started and was followed by a course of radiotherapy. By November 5, 1946, the patient's back pain was greatly relieved, but she continued to complain of stiffness in the hands and wrists. On July 12, 1947, the total blood protein was 5.8 Gm. per cent with albumin 3.2 Gm. per cent and globulin 2.6 Gm. per cent.

The patient is still in the hospital.

## CASE NO. 6

Patient M. K. (Hospital admission no. 110969), a 53 year old woman, was admitted on August 22, 1946 for complaints of severe pain in the lower back and right thigh of 18 months duration and inability to walk since November 1945.

Physical examination revealed gibbus of D-10 and percussion tenderness over lower thoracic spine

and iliac crest. X-ray studies showed numerous small radiolucent areas throughout the calvarium, partial collapse of upper costal plate L1, pathological fracture of L3.

Laboratory examinations showed total serum protein 7.5 Gm. per cent, albumin 3.9 Gm. per cent, globulin 3.6 Gm. per cent, hemoglobin 11.5 Gm. per 100 cc., red blood count 3,700,000 per cu. mm., white blood count 5,000 with normal differential. Bone marrow aspiration showed 50 per cent plasma cells typical of multiple myeloma. Urine was negative for Bence-Jones protein.

A first course of neostibosan was given from September 3 to September 20; a second course of neostibosan from October 11 through October 26, 1946, followed by radiotherapy. At the start of treatment, the patient was completely immobilized and unable to make the slightest movement because of pain. By November 26, 1946 the patient had considerable relief of pain; by December 26 she was able to move about freely in bed. By mid-February 1947, opiates and demerol previously necessary for relief of pain could be withdrawn. The patient is learning to walk with the aid of Taylor's brace. Repeated bone marrow aspiration of January 15, 1947 showed 28 per cent plasma cells.

Patient is still in the hospital.

#### CASE NO. 7

Patient A. C. (Hospital admission no. 11030), a 70 year old woman, was admitted on September 6, 1946 for pain in the neck of one year's duration, for the last months becoming so severe as to totally incapacitate the patient; also pain in the right thigh. The neck was extended and attempts at flexion met with cries of pain.

Laboratory studies showed: hemoglobin 35 per cent, red blood count 1,500,000, white blood count 5,000, with normal differential. Total serum protein 9.2 Gm. per cent, albumin 4 Gm. per cent, globulin 5.2 Gm. per cent, urea nitrogen 34 mg. per cent.

Bone marrow studies showed 58 per cent plasma cells in the sternal aspiration and 75 per cent in iliac crest aspiration. The urine was positive for Bence-Jones proteins. Because of severe pain in the neck a plaster collar was applied on September 16. Neostibosan treatment was started at the same time. A very marked relief of pain was obtained in a few days, so that the collar was removed. The pain in the neck has failed to recur. The pain in the back has also disappeared. The relief of the pain was so marked that no radiotherapy was given.

Because of persistent anemia with a hemoglobin below 50 per cent several blood transfusions were given to this patient.

Another course of neostibosan treatment was given from January 7, 1947 through January 22, 1947 at which time the patient showed difficulty in hearing which persisted for a month after the treatment had been stopped. On January 31, 1947, determinations of blood showed: total protein 7.1 Gm. per cent, albumin 3.1 Gm. per cent, globulin 3.5 Gm. per cent, urea nitrogen 21 per cent.

#### SUMMARY

This is a preliminary report on the treatment of 7 patients with multiple myeloma with antimony compounds. The therapeutic trial was based on the fact that multiple myeloma is frequently associated with hyperglobulinemia, a characteristic shared with other diseases in which antimony had been found therapeutically effective.

The results obtained thus far are insufficient to warrant conclusions, although they indicate a possible influence of antimony on myeloma tissue. This influence is seen mainly as an increased radiosensitivity when patients are subsequently treated with x-rays.

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# THE INFLUENCE OF NITROGEN MUSTARD ON MYCOSIS FUNGOIDES

## OBSERVATIONS RELATING ITS EFFECT TO THE RETICULO-ENDOTHELIAL SYSTEM

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THIS report deals with the therapeutic effect of nitrogen mustard on 6 patients with mycosis fungoides. The nitrogen mustards, i.e., the *N*-chloroethylamines, of which the methyl-bis form is being studied clinically, has been shown to be effective in the control of certain types of malignancies.<sup>2-4</sup> The effects of this group of chemicals, which appear to be on the nuclear structure of the cell, resemble most closely the action of short wave radiation rather than that of any known chemical reaction.<sup>1, 6</sup> The cellular susceptibility to nitrogen mustard is related to the proliferative activity of the tissue. Thus malignant tissue is more susceptible to its action than is normal tissue; while in the normal, the bone marrow elements, the lymph nodes, and probably the liver reflect the cytotoxic action of the mustards. This action may result in lymphopenia, granulocytopenia, thrombocytopenia and moderate anemia as well as nausea and vomiting.

Preliminary clinical experience with the methyl-bis-nitrogen mustard also indicates that a specific effect is exerted upon lymphatic tissues and upon malignancies of lymphatic origin. Nitrogen mustard has been found to be most effective in the treatment of Hodgkin's disease and lymphosarcoma, and less satisfactory in the treatment of the leukemias. Preliminary trials of the chemical in certain other neoplasms, viz., melanomasarcoma, metastatic mammary and cervical carcinoma, multiple myeloma and sympatheticoblastoma, have not been encouraging.<sup>3</sup> However, sufficiently favorable results have been obtained in carcinoma of the lung and in polycythemia vera to warrant further trial.

The release of this chemical for further clinical investigation under the auspices of the National Research Council has stimulated much interest in its effects upon malignancies, particularly of the lymphatic tissues. It was natural, therefore, that nitrogen mustard was tried in mycosis fungoides because of its relationship to diseases of the lymph nodes.<sup>5</sup> While the origin of the disease has been in dispute for nearly a century, the opinion most generally held is that it is a neoplasm originating in the reticulo-endothelium of the skin,<sup>7, 8</sup> classified, therefore, as a variety of the related diseases now grouped as lymphoblastomas. This group includes mycosis fungoides, Hodgkin's disease, lymphatic leukemia and the various forms of lymphosarcoma.

Mycosis fungoides was first described, under the name of *pian fungoides*, by Alibert in 1806. Later the name was changed to mycosis fungoides because of the development of mushroom-like tumors. Two types of the disease have been de-

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scribed. The first type, the most common, begins with superficial inflammatory eruptions known as premycotic lesions, and resemble eczema, psoriasis, parapsoriasis, or dermatitis exfoliativa. These premycotic lesions may be indistinguishable from the chronic dermatoses unless the infiltrates characteristic of the later stages of the disease are present. Intense itching is generally a prominent feature. These lesions may be persistent or recurrent. After variable and generally long periods of time, characteristic infiltrations of neoplastic cells appear in the lesions. This stage of infiltration is then followed, after months or years, by the formation of fungoid tumors. The second type, the so-called "Tumeurs d'emblée," is characterized by tumor development not preceded by the premycotic or infiltrative stages.

Heretofore, x-radiation of the lesions, either during the premycotic, infiltrative or tumor stage, has been the only therapeutic measure of proven benefit. Generally it induces involution of the lesions and relief from the intense pruritis. Sooner or later, however, the eruption becomes radio-resistant. The lesions then spread and may ulcerate; and the lymph nodes may become greatly enlarged. The pruritis becomes more intense. Death finally results from inanition or from intercurrent infections.

#### CASE REPORTS

*Case 1.* Mrs. R. G., a 67 year old white female, was first seen in the clinic in August 1945, complaining of a pruritic skin eruption of 10 years duration. This first appeared on her back and then spread to involve the entire body. The itching was characterized by remissions and exacerbations. Gradually, the skin became thickened and brawny. For several years prior to 1945 she received repeated courses of superficial x-ray therapy, with some temporary improvement each time. When first seen at this hospital, physical examination revealed that the skin of the body was markedly thickened, with numerous nodules and small areas of ulceration. There was no enlargement of spleen or lymph nodes. Skin biopsy revealed mycosis fungoides. The blood count was normal. During the next three months she was given filtered x-radiation in divided doses of 300R and 800R over the trunk with little improvement. During the following year she received sodium thiosulphate intravenously and diphtheria toxoid intramuscularly without benefit.

On October 31, 1946 she was admitted to the hospital for nitrogen mustard therapy. Physical examination at this time revealed an extensive papular, eczematoid eruption involving the entire body except the head, the palms and soles. The skin was thickened, dull red in color and covered with superficial excoriations (figure 1). There were a few discrete nodes in the cervical and inguinal regions. The ankles were slightly edematous.

*Laboratory examination.* The urine, Kline test, blood N.P.N. and uric acid determinations were normal. The blood count was R.b.c. 4.3 M., Hgb. 82 per cent (13.0 Gm.), W.b.c. 8,200 with polymorphs 67 per cent, eosinophils 3 per cent, lymphocytes 21 per cent and monocytes 9 per cent. The serum albumin was 3.0 Gm. per cent, and the serum globulin was 3.5 Gm. per cent. Sedimentation rate was 12 mm. in 21 minutes (Linzenmeier). The electrocardiogram was within normal limits. Sternal marrow puncture showed a slight increase of the mature eosinophils and the eosinophilic myelocytes. A biopsy of the skin was reported as follows: "Section of the skin reveals a moderate acanthosis. The epithelium in places is atrophic and an occasional small ulcer is seen. There is slight parakeratosis of the surface. The superficial portion of the corium and papillae shows striking infiltration by cells which vary considerably in size and shape. Many of the cells are plasma cells. There are also a number of lymphocytes. In addition, there can be seen large mononuclear cells with abundant cytoplasm and large vesicular nuclei. There are many dilated capillaries with prominent endothelial cells. Diagnosis: Mycosis fungoides" (figure 2).

*Treatment and course.* On November 11, 12 and 13, 1946 the patient was given 5 mg. of nitrogen mustard for a total of 15 mg. Under treatment the white count dropped from 5400 with P. 79 per cent and L. 17



FIG. 1. CASE 1 (R. G.) EXTENSIVE ECZEMATOID LESIONS PRIOR TO TREATMENT



FIG. 2. CASE 1 (R. G.) SKIN BIOPSY PRIOR TO TREATMENT, SHOWING EXTENSIVE INFILTRATION WITH LYMPHOCYTES AND RETICULO-ENDOTHELIAL CELLS

per cent on November 11, to 3,600 with P. 76 per cent and L. 14 per cent on November 19, 1946, a decrease in the lymphocytes from 918 to 504 per cu. mm. There was the usual toxic reaction with nausea and vomiting several hours after each injection. There was prompt response to therapy. Four days after the beginning of treatment, there was a definite involution of the eruption and marked reduction of pruritis. One week later the skin of the entire body was clear, except for some large inflammatory eczematoid patches extending across the right scapula and right breast (figure 3). At this time small groups of minute vesicles were noticed interspersed among the eczematous and infiltrated patches. The patient's only complaints were itching and pain (radicular in character) confined to these areas. X-ray examination of the spine on November 27, 1946 showed a compression of the fourth dorsal vertebra. A second biopsy of the skin was taken, immediately adjacent to the area of the first biopsy, on the ninth day after treatment was started. The pathological report is as follows: "The section shows epithelium of average thickness with slight keratinization. There are a moderate number of dilated capillaries in the superficial corium and

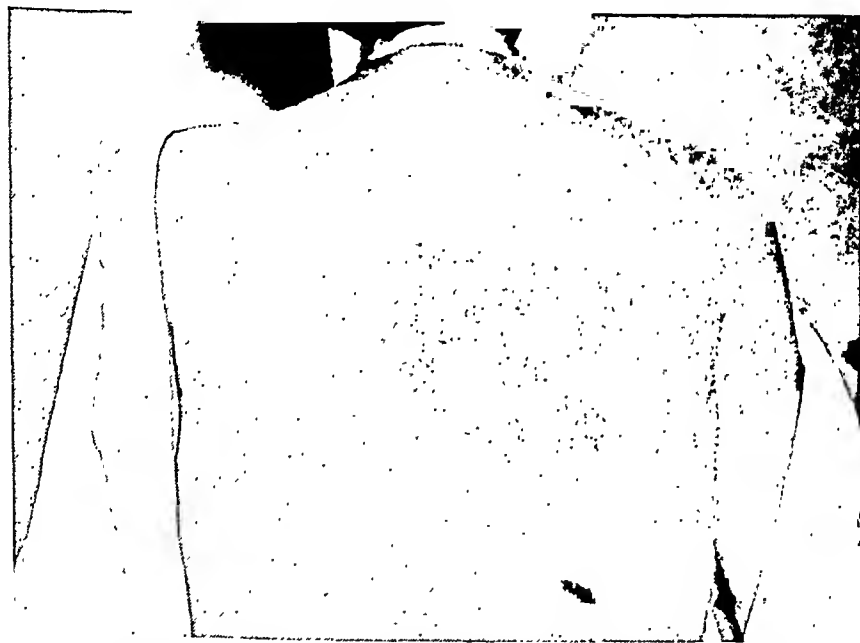


FIG. 3. CASE I (R. G.) 10 DAYS AFTER ONSET OF TREATMENT SHOWING COMPLETE CLEARING OF THE MYCOSIS FUNGOIDES LESIONS AND THE PERSISTING VESICULAR ERUPTION

papillae. A number of pigmented chromatophores are seen. There are a small number of lymphocytes and mononuclear cells located perivascularly. The intense infiltrate of the earlier sections is gone (figure 4). Diagnosis: Chronic dermatitis, slight."

During the months of December 1946 and January and February 1947, the patient remained free of symptoms. The radicular rash subsided considerably. However, in mid-February 1947, itching of the skin recurred. By February 26, 1947, about a dozen noninflammatory papules appeared on the forearm and left chest indicating a recurrence of the original disease. On April 2, 3 and 4, 1947, a second course of nitrogen mustard was given consisting of 6 mg. doses, for a total of 18 mg. By April 7, the new lesions had begun to disappear and by April 14, the skin had again become clear except for the previously noted radicular eruption. During this course of therapy, the lymphocyte count dropped from 1,560 (26 per cent of 6,000) to 240 (8 per cent of 3,000), 8 days after the onset of the treatment. By April 24, the count had returned to normal. When last seen on May 28 the patient was free of all lesions except for the ever present radicular lesion on the right breast and chest. The itching had completely disappeared.

*Comment.* This patient suffered extensive lesions of the body for 11 years. The lesions reacted to x-radiation at first but in recent years have been radio-resistant. Two courses of treatment with nitrogen mustard, totaling 33 mg., caused a disappearance of the infiltration of the skin and the pruritis. The skin now appeared normal except for a radicular herpetic lesion over the right mid-back and breast which is associated with x-ray evidence of a destructive lesion of the fourth dorsal vertebra. The patient remained clinically well for 3 months, following the first course and for 2 months following the second course to date of report.



FIG. 4. CASE 1 (R. G.) BIOPSY TAKEN 9 DAYS AFTER ONSET OF TREATMENT SHOWING DISAPPEARANCE OF THE INTENSE INFILTRATE

*Case 2.* Mr. R. H., age 72, had been followed in the clinic several years for a persisting pruritic, eczematoid eruption of 8 years duration. These lesions were present chiefly on the legs and thighs. During the past year many similar patches appeared on the trunk and upper extremities. Many of these had become thick and leathery. Concomitantly, several painless nodes appeared in the axillar and groin. On August 2, 1946 a biopsy taken from a lesion on the back was reported as showing "a massive infiltration of the cutis by reticulum cells causing obliteration of the rete pegs and atrophy or disintegration of the overlying epidermis. The lesion is compatible with mycosis fungoides as seen in late stages but lacks some of the features such as pleomorphism of cells, intra-epidermal focal cellular masses and clumping and giant cell formation. It is indistinguishable from lymphosarcoma of the skin." A sternal marrow procedure showed hyperplasia of the myeloid elements of the marrow. His past history, personal history and review by systems were noncontributory.

On January 7, 1947 he was admitted to the hospital for nitrogen mustard therapy. Upon physical

examination he presented a generalized eruption consisting of about a dozen coin to palm sized patches located on the upper back, the legs, the back of the hands and forearms. These patches were pink in color, scaly, fairly well margined and moderately thick and infiltrated. Pruritis was evidenced by superficial excoriations of all the lesions. There were several enlarged, firm, nontender and freely moveable lymph nodes in the axillae, posterior cervical and inguinal regions. These measured 2 to 3 cm. in diameter. The remainder of the physical examination was normal.

*Laboratory examination.* The blood count showed R.b.c. 5.0 M., Hgb. 93 per cent, W.b.c. 5,200 with P. 65 per cent, E. 2 per cent, L. 18 per cent and M. 15 per cent. The blood platelets were 135,270 per cu. mm. The Kline test was negative. The urine and the blood sedimentation rate were normal. Blood N.P.N. 32 mg. per cent, serum albumin 4.7 Gm. per cent, serum globulin 4.0 Gm. per cent. X-ray examination of the chest was normal.

*Therapy and course.* The patient was given 4 doses of nitrogen mustard of 6 mg. each intravenously on January 11, 13, 15 and 18, 1947. By January 20 there was a marked decrease in the itching, and a noticeable involution of the lesions. The skin became much softer. There was no significant change in the blood counts. The patient was discharged from the hospital on January 28, 1947 with only a faint erythema remaining at the sites of some of the lesions, and considerable shrinking of the enlarged lymph nodes. A repeat skin biopsy on January 21, 1947 showed some of the cells characteristic of mycosis fungoides but a marked decrease in the amount of cellular infiltration. Throughout the course of treatment, the blood counts remained unchanged. To date, 5 months after onset of treatment, he has remained well.

*Comment.* This patient has had mycosis fungoides for 8 years which was radio-resistant. The skin biopsy was unusual in that extensive infiltration of reticulum cells was found with but little pleomorphism. Twenty-four mg. of nitrogen mustard resulted in a clinical remission for the period of 5 months of observation.

*Case 3.* Mr. F. S., age 62, was admitted to the hospital on January 23, 1947 complaining of an eczematoid pruritic eruption of twelve years duration. The eruption first appeared on the hands and wrists later spreading to the legs and thighs. For the past 6 years the eruption has been generalized and intensely pruritic. Since the onset of his illness the patient has received a number of courses of superficial x-ray radiation. During the past 3 years x-radiation has produced only partial temporary remissions. For the past 6 months the lesions have been totally radio-resistant. Three months prior to admission to the hospital, a diagnosis of mycosis fungoides was made from a biopsy of the skin. In addition, during the past 3 years the patient has become progressively more dyspneic on exertion. In the week prior to admission he developed a productive cough.

*Physical examination.* The face, trunk, and all extremities were covered with scattered pink to red patches ranging in size from a silver dollar to saucer-like plaques. Some of the patches were scaly, some moist and eczematoid, and all were infiltrated. The skin of both legs was completely covered with these lesions. There was slight adenopathy in the cervical regions but not elsewhere. Liver and spleen were not palpable. The fundi showed grade I arteriosclerosis; the chest was emphysematous with increased resonance, diminished breath sound and numerous coarse rales. The heart was normal in size, but there was considerable ankle edema. The blood pressure was normal. The prostate was enlarged, but firm, smooth and nontender.

*Laboratory examination.* The R.b.c. varied between 6,170,000 and 5,950,000, Hgb. 114 per cent (19.3 Gm.) and the W.b.c., 7,400 with P. 76 per cent, E. 3 per cent, L. 11 per cent, M. 10 per cent. The sedimentation rate was 12 mm. in 324 minutes (Linzenmeier). Urinalysis and sputum studies were normal. The blood N.P.N. was 32 mg. per cent; the blood Wassermann and Kline tests were negative; the serum albumin was 4.0 Gm. per cent and the serum globulin was 4.0 Gm. per cent. X-ray of chest revealed an increase in bronchovesicular markings and some adhesions in both costophrenic angles, suggestive of a chronic bronchitis. The heart was normal in size and configuration. EKG showed left axis deviation and "myocardial impairment." Biopsy of the skin showed a moderate amount of acanthosis. The underlying corium showed scattered small groups of poorly defined cells, a moderate number of round cells, probably lymphocytes, and occasional large mononuclear cells, probably histocytes.

*Therapy and course.* Because of his chronic bronchitis, the patient was given penicillin aerosol, 75,000 units daily, from January 29 through February 7, 1947, with considerable improvement of his pulmonary symptoms.

On January 31, February 1, 3, and 4 he was given 6 mg. of nitrogen mustard intravenously for a total of 24 mg. By February 4, there was a great diminution in the pruritis and by February 9 there was noticeable involution of all the lesions with complete disappearance of the lesions on his arms. However, itching and a new type of papular eruption appeared on his lower extremities. This was diagnosed as a penicillin reaction and was associated with an eosinophilia of 4-13 per cent. During the nitrogen mustard therapy, the blood counts remained unchanged, but in the following 2 weeks there was a progressive drop in the leukocyte count to a level of 3000 with P. 64 per cent and L. 10 per cent.

By February 19 it was evident that the remaining lesions were not involuting. He was then given a second course of nitrogen mustard of 6 mg. each on February 24, 26, 28 and March 2. This was followed by a considerable reduction in the number and size of the remaining lesions with a corresponding reduction in the itching. On March 18 however, a slight recurrence was evidenced by the appearance of a few superficial pink spots. On March 25, 1947 he was readmitted for further treatment. His general condition was poor due primarily to an increase in his pulmonary and cardiac symptoms. He was markedly dyspneic and cyanotic and showed signs of right heart failure. He was digitalized with some improvement. Further nitrogen mustard was withheld. Instead he received spray x-radiation, 3 times weekly (for 3 weeks), 30R units on each side of his body, and urethane, 0.3 Gm., 3 times a day. After the third x-radiation he began to improve even though he had been radio-resistant previously. Itching was much relieved although still present. His cardiac status remained poor with dyspnea, cyanosis and orthopnea and he died on March 25, 1947 in congestive heart failure. No autopsy was obtained.

*Comment.* This patient had extensive premycotic lesions of the skin for 12 years which responded partially to x-radiation. For 6 months there was complete radio-resistance. Two courses of nitrogen mustard of 24 mg. each, totaling 48 mg. induced a rapid involution in the lesions and pruritis. Improvement was first noted on the fourth day of treatment. One and one-half months after the onset of treatment, relapse was noted. X-radiation, plus urethane, in spite of previous radio-resistance, was effective in controlling the disease. He was clear of lesions and relatively asymptomatic until the day of death  $3\frac{1}{2}$  months after the onset of therapy.

*Case 4.* Mrs. A. C., age 58, was admitted to the hospital on December 23, 1946. She had been under observation and treatment for mycosis fungoides at several clinics for many years. Her present illness began 30 years ago with an itching rash on the upper back. Similar patches appeared over the entire body during the next few years. The lesions resembled psoriasis, but 6 years ago a diagnosis of mycosis fungoides was established. Since then she had received x-radiation at various intervals with partial remissions. For the past year her disease had been radio-resistant. Upon physical examination she presented a universal eruption of coin to saucer-sized plaques, which were dull red, infiltrated and covered with moderately thick adherent scales. Many lesions were weeping (figure 5). The heart was slightly enlarged to the left, with a grade III blowing systolic murmur at the apex.

*Laboratory examination.* The R.b.c. was 4,300,000, Hgb. 79 per cent, W.b.c. 12,800 with P. 57 per cent, E. 14 per cent, L. 20 per cent, B. 2 per cent and M. 7 per cent. Urinalysis, blood Kline test, and total blood proteins were normal. A biopsy of the skin, showed the typical histological picture of mycosis fungoides, with a dense polymorphous infiltrate and scattered histiocytes.

*Therapy and course.* The patient received 3 injections of nitrogen mustard intravenously on December 24, 26 and 27, 1946 for a total of 18 mg. The blood count and differential remained unchanged. By December 30, there was a marked decrease in the infiltration of the patches. The scaling had greatly decreased. The color was now pink rather than a deep red, but the pruritis remained unchanged. On January 2, a repeat biopsy, taken from the same lesion, showed a considerable decrease in the number of cells comprising the infiltrate. A second course of therapy was begun 3 weeks later. On January 14, 17, 21 and 24, 1947, she received 4 injections of nitrogen mustard intravenously, for a total of 24 mg. On January 24, the white count was 3,000 and on January 28, it had dropped to 1200 with a normal differential count. By February 5, the white blood cell count had returned to 7,800 with a normal differential. The R.b.c., Hgb. and platelet counts were unaffected. Following the second course of treatment, there was a further decrease in the intensity of the erythema and infiltration in the patches and a complete disappearance of the exudation.

(figure 6). The pruritis still persisted, but was much less intense. A third skin biopsy on January 30, 1947, from the same patch, was reported as "mycosis fungoides with further decrease in cellular infiltration of the cutis." Six weeks later, itching became more intense. Some of the lesions on the arms and legs became more infiltrated and nodular. Superficial ulcerations involved some of the nodules on the left foot. The latter ulcerations progressed, coalescing to form a palm-sized area of painful ulceration on the instep of the left foot. On March 17, 18 and 20 a third course of nitrogen mustard was given with simultaneous



FIG. 5

FIG. 5. CASE 4 (A. C.) SHOWING EXTENSIVE INFILTRATED ECZEMATOID LESIONS PRIOR TO TREATMENT

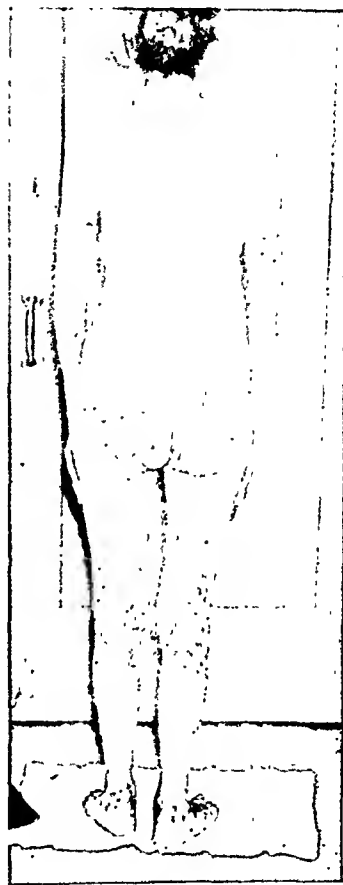


FIG. 6

FIG. 6. CASE 4 (A. C.) APPEARANCE FOLLOWING SECOND COURSE OF NITROGEN MUSTARD THERAPY, ONE MONTH AFTER THE ONSET OF TREATMENT

x-radiation to the ulcer. In the course of 3 weeks there was rapid healing of the ulcer with disappearance of pain.

By April 10, however, there was some recurrence of a generalized pruritis and a fourth course of nitrogen mustard consisting of 6 mg. doses was given on April 11, 12, 15 and 16 for a total of 24 mg. The pruritis and infiltration again improved. Following this, however, all elements of the blood were depressed. The R.b.c. dropped to 2,800,000, Hgb. 54 per cent, W.b.c. 250 with P. 42 per cent, E. 6 per cent, L. 44 per cent, M. 8 per cent, and platelets 40,000. The bleeding time was 4½ minutes. The tourniquet test was positive. A purpuric eruption was manifest. No secondary infection set in, but she was given penicillin,

800,000 units daily, prophylactically for about a week and 1500 cc. of whole blood. By May 15, the platelet count had risen to 145,000. On May 28, the W.b.c. had returned to a level of 2,800 with P. 33 per cent, E. 6 per cent, L. 31 per cent and M. 10 per cent. At the time of writing, the skin is generally somewhat indurated. Pruritis is mild. The ulcerations of the left foot have not returned.

*Comment.* This patient suffered from mycosis fungoides for 30 years. For 1 year her lesions had been radio-resistant. Four courses totaling 84 mg. of nitrogen mustard were given over a period of  $3\frac{1}{2}$  months. Each course of treatment was followed by a marked regression of the lesions. This, in turn, was followed in several weeks by a partial relapse. Six weeks after the second course a recurrence of the lesions was accompanied by painful ulceration of the foot. A third course of nitrogen mustard of 24 mg., together with x-radiation of the foot, produced healing of the ulcer with relief of pain. The fourth course of nitrogen mustard of 24 mg., together with x-radiation to the foot, produced healing of the ulcer with relief of pain. The fourth course of nitrogen mustard of 24 mg. was followed by a temporary pancytopenia. Five months after the onset of therapy, the patient has considerable symptomatic relief and about 50% clearing of her mycosis fungoides lesions. In this instance the combined use of nitrogen mustard and x-radiation of ulcers on her foot seemed to give reasonably good results.

*Case 5.* Mr. K. S., age 50, first noted an itching rash on his right leg  $2\frac{1}{2}$  years ago. This soon spread to his right hip. He was given sulfa drugs and penicillin without effect. Later, a biopsy established the diagnosis of mycosis fungoides. X-radiation was then given with almost complete remission for 6-7 weeks, until about October 1945. These recurrent lesions were acute and fulminant in character, commencing as vesicles and bullae that rapidly became infiltrated. The infiltrated lesions then assumed a nodular and ulcerated character. Since October 1945 he received large amounts of x-radiation with little effect. He also received arsenic without benefit.

On January 18, 1947 he was admitted to the hospital for study and treatment. Physical examination was normal, except for the extensive lesions of the skin. X-ray showed the chest was normal. The trunk and extremities were covered with numerous annular, serpiginous, infiltrated lesions which had a tendency to clear in the center and spread peripherally. Some involved fairly large plaques of skin, with central clearing, and with tendencies toward coalescence. On the left hand, in the upper right groin and on the feet, extensive sloughing of the skin had taken place leaving large areas of denudations and deeper ulcers. In the right groin the area of denudation measured about 12 by 6 inches. The right foot was completely denuded over the anterior  $\frac{2}{3}$  of the foot and toes leaving an intensely red and painful ulcer that was secondarily infected. A somewhat smaller area of the left foot was similarly involved. These lesions were intensely pruritic.

*Laboratory examination.* Urinalysis was normal. R.b.c. 4,020,000, Hgb. 11.8 Gm., W.b.c. 7,700 with P. 66 per cent, L. 20 per cent, M. 12 per cent, E. 3 per cent and B. 1 per cent. The smear was normal and the platelets present in normal amounts. Biopsy of a skin lesion confirmed the diagnosis of mycosis fungoides, but showed few specific changes.

*Treatment and course.* The patient was given a concentrated course of nitrogen mustard consisting of 7 mg. per dose on January 18, 19, 21, 24, 25 and 27, a total of 42 mg. By the fifth day of treatment there was marked decrease in the number and size of the lesions and in the intensity of itching. The lymphocytes dropped from 1,540 at the onset of treatment to 462. Upon completion of the course of therapy the extensive lesions had almost completely disappeared. At this time there were only several scattered annular lesions about the trunk. The denudations of the left foot and right thigh had healed completely. The right foot, which was the most extensively involved, showed considerable, but not complete healing. New areas of skin began to appear and the secondary infection cleared without other therapy. Two weeks later, however, reactivation of the disease was evidenced by a dozen or so tiny new lesions and an increase in exudation over the pre-existing ones. By February 26, the new lesions had become faint, large and all lesions were oozing considerably. He was given one injection of 7 mg. of nitrogen mustard on

February 19 with the thought of trying weekly "suppressive" doses. By February 26, the progression of the lesions demanded a full course of treatment. On February 26, 27 and 28 and March 1, 3 and 4 he received a second course of 8 mg. doses for a total of 48 mg. The lymphocyte count dropped from 1,800 (30 per cent of 6,000) to 225 (11 per cent of 2,050 on March 5) which forced the abandonment of heavier therapy. By March 2, the lesions began to regress. There was steady improvement and by March 10 about half the lesions had disappeared and the remainder had regressed to at least one-half their original size. The denuded area of the right foot remained but was covered with patches of new skin. He was able to walk without pain in his foot for the first time since the onset of this ulceration. This improvement was short-lived. In a few days the ominous signs of recurrence, namely an increase in oozing, appeared. He continued to regress rapidly and was readmitted to the hospital on April 10, 1947 for further treatment. At this time the entire body was covered with circinate, crusted lesions, and the epithelium of both feet and hands was extensively denuded. His admission blood count showed R.b.c. 3,366,000, Hgb. 58 per cent, W.b.c. 5,000, P. 68 per cent, E. 1 per cent, B. 0.5 per cent, L. 17.5 per cent, and M. 12 per cent. The platelets were present in average numbers. He was given a third course of 6 doses of 7 mg. each of nitrogen mustard on April 11, 12, 15, 16, 19 and 21. To this was added colchicine gr. 1/100 3 times a day in an attempt to augment the effect of nitrogen mustard on the nuclear disintegration. The colchicine was discontinued after one week due to severe nausea, vomiting and diarrhea. On April 19, total body radiation, 30 R to each side, was begun 2 times weekly. By May 2, the skin lesions showed a tendency to heal, but by May 10 they began to recur. On May 16 the white count dropped to 600 with P. 58 per cent, E. 4 per cent, L. 18 per cent, and M. 20 per cent. The platelet count was 260,000. X-ray treatment at this time was discontinued. He began to show a septic temperature curve. Penicillin, 800,000 units daily, was given. Blood cultures prior to the administration of the penicillin showed no growth. Ulceration of the skin lesions extended rapidly. His general condition deteriorated and on May 22 signs of bronchopneumonia appeared. His W.b.c. on May 28 was 1,600 with P. 54 per cent, E. 3 per cent, L. 32 per cent and M. 10 per cent. On May 30, he appeared moribund with a temperature of 104 degrees and very extensive skin involvement with ulceration. Death occurred on June 4.

*Comment.* This 50 year old male gave a history of mycosis fungoides of only 2½ years duration, with early radio-insensitivity. His skin lesions were unusual in that they started as bullae and vesicles and rapidly reached the tumor stage with extensive ulceration. One hundred and thirty-two mg. of nitrogen mustard were given in 3 courses over a period of 3 months. The last course was combined with colchicine and x-radiation. Each course was followed by a partial remission of the disease, but each remission was lesser in degree and duration than the previous one. Finally, the disease process outran the effectiveness of the treatment. In this case the nitrogen mustard must be considered a failure.

*Case 6.* Mr. C. A., age 54, was admitted to the Los Angeles County General Hospital October 21, 1946, because of a generalized itching eruption and painful ulcers on the lower abdomen and genitals. His present illness began fifteen months earlier with an itching rash on the back, spreading to the extremities. Six months later a similar eruption appeared on the lower abdomen, pubis and genitals. Two months later the genital lesions ulcerated.

*Physical examination.* Upon examination, he presented a generalized eruption consisting of large erythematous patches and plaques. Some of the patches were elevated and infiltrated, others were eczematous and those over the suprapubic areas and scrotum were covered with multiple ulcerated nodules (figure 7). the remainder of the examination was normal.

*Laboratory examination.* The hemogram showed: R.b.c. 3,940,000, Hgb. 13.4 Gm., W.b.c. 6,400, P. 62 per cent, L. 18 per cent, M. 13 per cent, E. 1 per cent, B. 2 per cent, promyelocytes 1 per cent, unclassified (young) 3 per cent, and platelets, normal. Biopsy from a nodule on the abdomen was reported as typical of mycosis fungoides and was characterized chiefly by an infiltration of lymphocytes. The Wassermann and Frei tests were negative.

*Therapy and course.* During the first week of November 1946, he received a total of 225R units of x-radiation to the eczematoid weeping plaques on the neck, and to the suprapubic area. No change was noted

during the following 3 weeks. On December 11, 13 and 15, he received 4.5 mg. nitrogen mustard, intravenously for a total of 13.5 mg. Daily blood counts showed no appreciable change. On December 16, the day after the last injection, he developed an acute inflammatory dermatitis of the right cheek in and around some small infiltrated nodules. This was thought to be a cellulitis although there was no elevation of temperature, white count, or acute adenitis. Penicillin, 320,000 units daily, was administered for 6 days. Ten days following the last injection of nitrogen mustard, all the ulcers were completely healed, the raised infiltrated plaques were flattened and dull brown in color, many of the flat eczematous patches had completely disappeared and the patient was greatly relieved of his pruritis (figure 8). However, the cellulitis-like eruption of the cheek was still present but not as acutely.

By January 10, the acute dermatitis of the cheek had subsided. However, the infiltrated plaques on the right elbow and forearm had become nodular and eczematous, and several new ulcers had appeared in the scrotum and in the groin. Three more intravenous injections of nitrogen mustard, 6 mg. each, were ad-



FIG. 7

FIG. 7. CASE 6 (C. A.) MYCOSIS FUNGOIDES NODULES AND ULCERS



FIG. 8

FIG. 8. CASE 6 (C. A.) COMPLETE HEALING OF ULCERS, AND INVOLUTION OF NODULES TEN DAYS AFTER THERAPY

ministered on January 11, 12, and 13, 1947. There was an immediate recurrence of the cellulitis-like dermatitis of the right cheek, with a similar acute eczematous dermatitis of the left side of the neck. Therapy was discontinued. One week later the ulcers were completely healed, and the infiltrated and eczematous plaques of the body greatly improved. The patient remained improved for the next 2 months. During this period the acute lesion on the cheek became infiltrated, thickened and nodular. A second biopsy showed the same characteristic infiltration of mycosis fungoides. By the end of the second month infiltrated nodules and new ulcers had appeared. He was given a third course of nitrogen mustard totaling 24 mg. Simultaneously, he was also given colchicine 1/100 gr. (0.6 mg.) 3 times a day increasing to 1.2 mg. One week after the course of nitrogen mustard therapy, the ulcers were again completely healed, the larger nodules had shrunk considerably in size, but the lesion on the cheek remained unchanged. This time improvement persisted for only 3 weeks. At the date of report he has begun to show early signs of recurrence.

*Comment.* This patient presented a rapidly progressive mycosis fungoides of 15 months duration, with multiple, large, eczematous, infiltrated patches and plaques, infiltrated nodules, and multiple ulcerated nodules on the lower abdomen, pubis and scrotum. He was given 3 courses of nitrogen mustard totaling 55.5 mg. The immediate response to the nitrogen mustard was excellent, but each course was followed shortly by a recurrence. A fixed drug eruption resembling cellulitis followed the first 2 courses of the drug, but not the third. Two courses of x-radiation were also given without effect. Colchicine was given along with the third course, but did not seem to be of value. The inability to arrest the disease process for more than a few weeks at a time stamps this case as a therapeutic failure.

	Case					
	1 (R. G.)	2 (R. H.)	3 (F. S.)	4 (A. C.)	5 (K. S.)	6 (C. A.)
Age.....	67	72	62	58	50	54
Duration of disease (yrs.)	10	8	12	30	2½	1½
Radio-insensitive.....	Several	Several	Several	1	1½	1½
Lesions.....	Eczematoid nodular	Eczematoid infiltrative	Eczematoid infiltrative	Eczematoid infiltrative	Tumors, ulcers	Tumors, ulcers
Biopsy findings	Large no. R-E cells	"massive" R-E cells	Moderate R-E cells	Dense	Minor changes	Abundant polymorph. infiltrate
N-Mustard dosage (mg.)	3 x 5	4 x 6	4 x 6	3 x 6	6 x 7	3 x 4.5
	3 x 6		4 x 6	4 x 6	6 x 8	3 x 6
				3 x 6 + x-ray 4 x 6	6 x 7 + x-ray	4 x 6
Total (mg.)....	33	24	48	84	132	55.5
Months observ.....	6	5	3½	5	4	3½
Results.....	Good	Good	Good	Fair	Death	Poor

## DISCUSSION

In each of the 6 cases studied, the mycotic lesions, irrespective of type or the stage of the disease, showed a marked immediate response to nitrogen mustard therapy. In each instance, the disease was radio-resistant. In 4 (cases 1, 2, 3, 4), the disease was of long duration (between 8 and 30 years), and had responded to x-radiation over an appreciable period of time. The radio-resistant phase of the disease had existed, at the time of study, for several years. In 2 instances (cases 5 and 6) the disease process was of short duration (2½ years and 15 months respectively). Here the disease was exceptionally acute, and radio-resistance existed almost from the onset. Hence, the response of the tumor cells to nitrogen mustard seems to bear no relationship to their resistance to x-radiation or the duration of the disease. In this the reaction of the lesions of mycosis fungoides parallel those of Hodgkin's disease.

The degree of response varied from case to case. By a strange twist of fate, the cases were seen (and numbered) in almost the exact order of their response to the drug. (See table above.) Cases 1, 2, 3 may be said to have had an excellent response.

In each instance the lesions more or less completely cleared and the itching was greatly relieved. Case 4, in which the disease has existed 30 years, has shown only partial improvement. Cases 5 and 6 must be classed as clinical failures. While there was a prompt response to the drug, each course was followed by a relapse within several weeks, and the duration of improvement became shorter and shorter. Case 5 died as a direct consequence of the disease; while case 6 is progressing steadily to the same end, his disease process having been unrestrained by any method at our command. These 2 cases are exceptional because of their short duration ( $2\frac{1}{2}$  and  $1\frac{1}{4}$  years), the rapid spread of the disease, and the biopsy findings as noted below.

The first 4 cases which showed good clinical responses, were of a similar nature. Their lesions were characterized by a prolonged premycotic stage (eczema, psoriasis, parapsoriasis) and infiltrations and nodule formation. There was little tendency toward breakdown into ulcerations or denudations. In contrast, cases 5 and 6 showed a distinctly different type of clinical picture, characterized by early nodule formation and extensive ulceration. In addition, case 5 presented the unusual feature of the lesions developing with vesicles and bullae which rapidly became infiltrated.

From a study of the biopsies, one fact has emerged which may be of considerable significance if substantiated in other types of clinical material. In cases 1, 2 and 3, which showed the best response, the biopsy specimens showed a high incidence of reticulo-endothelial cells. In case 2 "a massive infiltration by reticulum cells" was commented upon. In case 1, there was a massive polymorphous infiltration including reticulo-endothelial cells. In case 3 there was moderate infiltration of lymphocytes and some reticulo-endothelial cells. These findings showed a direct correlation with the clinical improvement. Thus, of the 4 cases, case 2 has done the best, clinically, case 1 second best, case 3 third best and case 4 the poorest. In contrast, cases 5 and 6 showed, on biopsy study, different pictures. In these 2 patients the histological picture presented an infiltrate in which the distribution and polymorphism was characteristic of mycosis fungoides, but sparse in reticulo-endothelial cells.

These observations conform with the recognized fact that Hodgkin's disease, presumed to be of reticulo-endothelial origin, responds better to the nitrogen mustards than do the lymphosarcomas or lymphatic leukemia which are more specifically diseases of the lymphoid apparatus. Of interest are the observations of one of us (H.H.H.) in 2 cases of monocytic leukemia and in one case of reticulum cell sarcoma. In the first case of monocytic leukemia, life was prolonged for several months beyond the expected date of exitus, and the blood monocytes were reduced at will by the nitrogen mustard. In the second case, the blood monocytes are reduced with great ease by the drug. In the case of the reticulum cell sarcoma a most remarkable effect was secured. The patient (E.F., aged 34, to be reported later) was semimoribund at the time of treatment. He presented generalized glandular enlargement of moderate degree and a splenomegaly. Nitrogen mustard therapy resulted in disappearance of all evidence of the disease process. At the date of writing, 6 months later, the only evidences of disease are a slight immaturity of the

blood lymphocytes and a somewhat greater than normal fatigue on exertion. These observations suggest that the point of attack of the nitrogen mustard is specifically on the reticulo-endothelial tissue. Considerable interest attaches to the effects of the nitrogen mustard in the reticulo-endothelioses and in other diseases in which this system is extensively involved. However, one case of widespread Kaposi's sarcoma (idiopathic, multiple, hemorrhagic sarcoma of Kaposi) which some investigators believe to be of reticulo-endothelial origin, was treated with nitrogen mustard by one of us (B.A.N.) without effect upon the disease.

#### SUMMARY AND CONCLUSIONS

1. Six patients with mycosis fungoides, resistant to x-radiation, have been treated with nitrogen mustard.
2. Each patient showed a striking immediate response to the drug.
3. The extent of the remissions of the disease process, during the period of study (from 4 to 7 months), varied with the nature of the lesions and the biopsy findings:
  - (a) Three patients with histories of prolonged premycotic stages and reticulo-endothelial cell hyperplasia on biopsy showed an excellent response to the drug.
  - (b) One patient with a history of prolonged premycotic stage and biopsy findings of a dense polymorphic infiltrate, but with sparse numbers of reticulo-endothelial cells showed a partial response to treatment.
  - (c) Two patients whose disease was of short duration with early nodule formation and ulceration with biopsy findings of rather few endothelial cells among the infiltrate showed rapid recurrences and progression of the disease.
4. Nitrogen mustard is an effective agent in the palliative treatment of mycosis fungoides.
5. It is suggested that the point of attack of the nitrogen mustard is on the neoplastic cell arising from the reticulo-endothelial system.
6. The response of mycosis fungoides to nitrogen mustard presents additional evidence of the neoplastic nature of the disease.

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# LECITHIN AND THE ERYTHROCYTE FACTOR IN THE BLOOD SEDIMENTATION PHENOMENON

By JOHN S. HIRSCHBOECK, M.D.

THE phenomenon of erythrocyte sedimentation in altered physiological states has intrigued physicians since the time of Hippocrates. It was the basis for the humoral theory of disease, which served as the official code of Western medicine until the advent of Virchow's cellular pathology. Fahraeus rediscovered the phenomenon of erythrocyte sedimentation in 1918, after it had been forgotten for over fifty years, and explained much of its physiology in the light of modern laboratory methods. He proved that the speed of erythrocyte sedimentation is proportional to the degree of rouleau aggregation among the erythrocytes. The sedimentation follows Stoke's Law, which is expressed by the following formula:

$$v = \frac{2}{9} g \frac{S - S_1}{\mu} r$$

$v$  = velocity of sedimentation

$g$  = gravity

$S$  = the density of the particle

$S_1$  = the density of the fluid

$\mu$  = the absolute viscosity of the fluid

$r$  = the radius of the particle

In blood all of the factors remain constant except the radius of the particle, e.g., the radius of the aggregations of red cells. The greater the rouleau aggregation the greater is  $r$  and the more rapid is the velocity of fall.<sup>1</sup> Ponder<sup>2</sup> has re-examined Fahraeus' thesis and confirmed it in all respects.

The explanation of rouleau aggregation is still quite uncertain. Why the non-motile erythrocytes should under certain conditions align themselves, one upon the other, in an orderly fashion like a stack of coins, remains one of the fascinating problems in hematology. Practically all investigators agree that it is ultimately a problem in physical chemistry. Thygesen<sup>3a</sup> has recently reviewed the literature and studied the problem exhaustively. The great majority of investigators are in agreement with Fahraeus when he states, "The chief cause of this phenomenon is an increase in amount of the easily precipitated protein fractions, the serum globulin or the fibrinogen or both."<sup>3</sup>

The ultimate basis for this statement is the demonstration that the erythrocytes settle out rapidly in solutions of fibrinogen and to a less extent in globulin and not at all in albumin. If blood is defibrinated the sedimentation is very slight; whereas the same blood with the fibrin still in it might have a very rapid sedimentation rate. Erythrocytes suspended in isotonic salt or Ringer's solution do not form rouleaux and do not settle out. Fahraeus, and with him Gram,<sup>4</sup> Bendix and

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Snapper,<sup>4</sup> Greisheimer et al.,<sup>5</sup> Westergren,<sup>6</sup> Ham and Curtis,<sup>7</sup> and Kylin,<sup>8</sup> therefore conclude that changes in the plasma proteins are responsible for the phenomenon. Kürten,<sup>9</sup> Alfred-Brown and Munro,<sup>10</sup> Nitschke,<sup>11</sup> Oelkers and Ohnesorge,<sup>12</sup> Bauer, Rossmesl, and Ropes,<sup>13</sup> Bing and Jessen,<sup>14</sup> and Kopp<sup>15</sup> believe that there are other factors which act either alone or together with the proteins.

Kürten<sup>9</sup> has attempted to show that the phenomenon depends upon changes in blood lipoids, notably cholesterol and lecithin. His interpretation received considerable acceptance in the chemical literature and among many who were unfamiliar with the work of Fahraeus. At the present time very little consideration is given to the plasma lipoids as factors in blood sedimentation, particularly since the thorough studies of Westergren, Thøerell, and Widstrom.<sup>6</sup>

Some investigators believe that fibrinogen is the most important factor. Ham and Curtis<sup>7</sup> go so far as to use the Rourke-Ernstene method of determining the sedimentation rate together with a mathematical constant as a method for the quantitative measurement of plasma fibrinogen. Alfred-Brown and Munro<sup>10</sup> and Ropes, Rossmesl, and Bauer<sup>13</sup> have demonstrated that fibrinogen may be just one factor and that other globulin fractions may be in certain cases as active as the fibrinogen. A fraction of fibrinogen isolated from rapidly sedimenting blood by Morrison<sup>13a</sup> has been found to be very active. This fraction, which he calls contractinogen, is salted out of plasma at a 16 $\frac{2}{3}$  per cent saturation with ammonium sulfate. Even when both the fibrinogen and the globulin are accounted for, an unexplained additional factor may occasionally be present to affect the sedimentation.

Practically all previous studies of blood sedimentation have dealt with the plasma factors alone. That the erythrocytes themselves may vary in their response to the plasma factors was first demonstrated by Frimberger.<sup>16</sup> He showed that the rouleau formation of erythrocytes suspended in standard artificial plasmas was quite constant except in certain diseases, notably diabetes. If the rouleaux are a physical and a chemical phenomenon, the erythrocytes must possess some factor which allows them to be so aggregated under the influence of the plasma. If the erythrocyte membrane is destroyed or altered in certain ways rouleaux do not form. It is the purpose of this investigation to examine the nature of this erythrocyte factor.

It has been known since 1836<sup>1</sup> that the addition of a gum or hydrophilic colloid to blood greatly increased the rouleau formation. Linzenmeyer<sup>17</sup> has studied the effect of gelatin and gum arabic on the sedimentation rate. Fahraeus suspended washed erythrocytes in solutions of sodium caseinate and demonstrated that this protein caused rouleau agglutination and augmented the sedimentation. Wiltshire<sup>18</sup> noticed that washed erythrocytes suspended in solutions of gum arabic, gum tragacanth, and gelatin formed rouleaux without the presence of any native plasma. Gelatin is a relatively standard material and if identical solutions are made from the same stock they will have identical properties. Standard artificial rouleau-forming plasmas of great constancy can therefore be prepared from time to time out of a single large stock of gelatin. Obviously these solutions will not compare perfectly with solutions made from other stocks of gelatin derived from different or even similar sources.

The concentration of erythrocytes in the plasma has a great influence on the sedimentation rate.<sup>3</sup> The lower the concentration the more rapid is the sedimentation. In order to make this factor constant it is merely necessary to centrifuge the blood for twenty minutes at a high speed and remove or add plasma to standardize the erythrocyte-plasma ratio. This same method may be used when working with washed erythrocytes and artificial gelatin plasmas.

If washed erythrocytes are suspended in standard gelatin solutions in constant concentration any variations in the sedimentation rate must necessarily be due to differences in the erythrocytes themselves. By using this method of investigation it is possible to discover variations in what may be called the erythrocyte factor in blood sedimentation.

#### TECHNIC USED IN INVESTIGATING THE ERYTHROCYTE FACTOR

Blood was removed with a syringe from veins of healthy men and women, of men and women with various diseases, of dogs, and of rabbits. Horse blood was obtained as it flowed from the jugular veins of dying horses slaughtered at an abattoir. Guinea pig blood was removed by cardiac puncture and turtle blood was collected as it flowed from the neck vessels of a recently decapitated animal. In all cases, in order to prevent coagulation, the blood was either placed in 3.8 per cent sodium citrate solution 4:1 or in vessels containing small amounts of sodium or ammonium oxalate. A quantity of this blood, usually 5 cc., was then centrifuged for 20 minutes in graduated hematocrit tubes, the supernatant plasma was removed, and 0.85 per cent sodium chloride solution was added to the erythrocytes to the capacity of the tube (15 cc.). After again agitating the tube and centrifuging for 20 minutes, the saline was removed and another volume of saline was added and the process repeated. The washing was repeated a second time, and in some experiments more often. The erythrocytes were then suspended in solutions of 1 per cent gelatin in 0.85 per cent sodium chloride to a concentration of one-third erythrocytes and two-thirds plasma. The suspensions were well mixed and then aspirated into Westergren sedimentation tubes. The readings, in terms of millimeters of settling, were made at the end of one hour.

#### VARIATIONS IN THE ERYTHROCYTE FACTOR IN HUMAN BLOOD

The blood was obtained from healthy and diseased men and women. Table 1 shows the erythrocyte factor compared with the sedimentation rate of the various samples of blood. In each case the erythrocytes were prepared in the manner previously described.

It is evident from table 1 that there may be variations in the erythrocyte factor among individuals. The majority of the cases were in the range between 120 and 130 mm. However, 2 normal males were definitely below this level. These results might explain the failure to correlate absolutely the quantitative plasma fibrinogen and globulin levels with the sedimentation rate. Those bloods which have a greater sedimentation rate than one would expect from the amount of fibrinogen and globulin in their plasma may be explained by the presence of a very strong erythro-

cyte factor, and those which should have a more rapid rate, by the presence of a very weak erythrocyte factor.

Frimberger<sup>16</sup> has made a similar study and did not find a great difference in the erythrocyte factor in a short series of cases. One patient with diabetes had a definitely weak erythrocyte factor.

If erythrocytes are suspended in hypotonic salt solutions they increase in size and become spherocytes as they imbibe water. In hypertonic salt solutions they lose their biconcave form and appear as shrunken spheres studded with barbs and are designated then as crenocytes. The influence of changes in the shape of the erythro-

TABLE 1.—*A Comparison between the Sedimentation of Blood and the Sedimentation of Erythrocytes from the Same Blood in a Standard Artificial Plasma*

Description of case	Erythrocyte factor (sed. rate of washed erythrocytes in 1% gelatin in saline)	Blood sedimentation rate (Westergren)
	mm. per hour	mm. per hour
Normal male.....	74	8
Normal male.....	78	3
Normal male.....	119	2
Normal male.....	122	6
Pregnant female.....	122	50
Chronic salpingo-oophoritis..	123	13
Normal female.....	125	4
Pregnant female.....	129	10
Pregnant female.....	130	45
Primary syphilis female ..	132	11

TABLE 2.—*The Erythrocyte Factor Is Not Altered by Changes in the Shape or Water Content of the Erythrocytes*

Solution	Without 1% gelatin	With 1% gelatin
	mm. per hour	mm. per hour
0.65% NaCl.....	2	126
0.85% NaCl.....	2	122
1.25% NaCl .....	2	124

cyte on the rouleau-forming tendency has been partially studied by Frimberger.<sup>16</sup> He demonstrated that hypotonicity or hypertonicity has no effect on the sedimentation rate in artificial plasmas. The results of a similar experiment are given in table 2.

The rouleaux which form have a very unusual appearance. In hypotonic solutions with 1 per cent gelatin the spheroid cells line up in rows and the appearance of the aggregation is more like a compressed chain of streptococci than a true rouleau. In hypertonic solutions with 1 per cent gelatin the crenated erythrocytes also endeavor to form true rouleaux, but because of the shape and the rigidity of the cell only a few erythrocytes manage to remain aligned in a row. Instead the adhesion of the cells to each other is multiplanar and a clump-like type of agglu-

tionation supervenes. In spite of the great difference in appearance and form of the agglutinated masses of cells in solutions of different concentrations of sodium chloride, the degree of agglutination as measured by the sedimentation rate is surprisingly constant. Table 2 also serves to demonstrate the role which gelatin plays in the agglutination inasmuch as the cells suspended in saline solutions alone manifest only an insignificant sedimentation of 2 mm. in one hour.

The shape of the erythrocyte is naturally altered into very bizarre forms in pernicious anemia. Poikilocytes of this type, pathologic in form, sediment normally. The general form of the rouleau is normal, but on close inspection it appears more like a compressed stack of figs than a stack of coins. Pernicious anemia poikilocytes when washed in saline maintain their shape and when suspended in gelatin solution settle out at the same rate as normal erythrocytes (table 3).

Changes in the erythrocyte shape in the direction of spherocytosis caused by hemolysins are not comparable to the changes produced by hypotonic saline.<sup>18</sup> The hemolysin actually alters the surface of the cell and interferes with normal surface effects produced by the plasma, and rouleau formation does not take place,

TABLE 3.—Comparison of the Erythrocyte Factor of Pernicious Anemia Poikilocytes with Normal Cells

	One-half hour	One hour
	(Wintrobe method)	
	mm.	mm.
Poikilocytes.....	32	46
Normal erythrocytes.....	35	46

(Washed cells were suspended in 1% gelatin in 0.85% NaCl solution.)

whereas with a hypotonic solution the surface is not changed chemically but is merely placed under a stretch.

Fahraeus observed many years ago that when blood with a rapid sedimentation rate was kept at 37 degrees C. for six hours without being moved, it lost its rouleau-forming power and consequently its rapid sedimentation rate. If, however, it was agitated during the period of incubation, as it is naturally while circulating within the body, the blood maintained its rouleau-forming power and settled out at practically the same rate as before. This unusual behavior remained unexplained until 1937, when Bergenheim and Fahraeus<sup>19</sup> demonstrated that an enzyme, lysolecithinase, converted lecithin into lysolecithin while the blood was static. The lysolecithin acts upon the erythrocyte surface as a hemolytic agent, making the cells incapable of forming rouleaux. Agitation of the blood interferes with the action of the enzyme and keeps the blood relatively normal.

Melgren,<sup>20</sup> Stephens,<sup>21</sup> and Gripwall<sup>22</sup> have attempted to explain the normal hemolysis going on in the spleen by the action of lysolecithinase. Knisely<sup>23</sup> demonstrated that the splenic sinusoids may act as reservoirs holding erythrocytes for as long as 12 hours. The spleen then may act as an incubator and allow the plasma lysolecithinase to form lysolecithin and begin the hemolysis of the static erythrocytes stored in the sinusoids. Both Melgren<sup>20</sup> and Stephens<sup>21</sup> have shown that the

sedimentation rate of blood from the splenic vein is slower than that from the splenic artery. Stephens also demonstrated that the settling erythrocytes became stratified. The prehemolyzed cells fall more slowly than the normal cells and remain above them as a semiopaque layer. There may be several strata, depending upon the degree of hemolysis in the spleen. This same phenomenon may occur in acquired and congenital hemolytic anemias. Gripwall<sup>22</sup> has attempted to explain hemolytic anemias as the result of an overactive reservoir function of the spleen with increased lysolecithin formation. Singer<sup>21</sup> has shown that there is a difference in susceptibility of erythrocytes to lysolecithin hemolysis. The red cells of congenital hemolytic anemia are very weak in their resistance to lysolecithin hemolysis while the cells of acquired or symptomatic hemolytic anemia have a normal resistance to lysolecithin hemolysis.

It is apparent that the erythrocyte factor is destroyed by lysolecithin. This substance is lecithin with the second unsaturated fatty acid radical replaced by a hydroxyl group.<sup>25</sup> The enzyme lysolecithin is apparently identical with the hemolytic agent of cobra venom.<sup>26</sup>

Saponin is a very effective hemolytic agent and is well suited to hematologic investigation because of the small amounts which can be used. Hintregger<sup>27</sup> ob-

TABLE 4.—*The Effect of Saponin on the Blood Sedimentation Rate*

	Washed human erythrocytes in 1% gelatin in 0.85% saline	Washed human erythrocytes in 1% gelatin in 0.85% saline with 0.2% saponin
	mm.	mm.
Sed. rate (one-half hour) . . .	122	0

served that the addition of saponin to normal hog blood in a concentration of 0.03 per cent reduced the sedimentation rate by 60 per cent. Table 4 shows the effect of adding saponin (Merck) in a concentration of 0.2 per cent to washed erythrocytes in artificial gelatin plasma. The erythrocytes were not hemolyzed until 24 hours had elapsed, but during the period within 10 minutes after adding the saponin, the cells had become spherical and failed to form rouleaux. The saponin apparently injures the erythrocyte surface in a manner similar to lysolecithin and interferes with rouleau agglutination.

The effect of autohemolysis on rouleau formation at low temperatures has not been studied to any extent. Blood may be stored from three to six weeks in a refrigerator without appreciable hemolysis taking place, especially when the blood is enriched with dextrose. One would expect that blood so preserved could maintain its rouleau-forming power. Table 5 shows the effect of storage on the erythrocyte factor in a specimen of dog blood. The animal was sacrificed with ether and as much blood as possible was collected in 3.8 per cent sodium citrate solution. It was kept thereafter at 5 degrees C. Slight hemolysis was present during the entire storage period. From the fourth to the seventeenth day, considerable free hemoglobin was released and the plasma had a definite red color. The blood was not tested for sterility and it is possible that the hemolysis may have had a bacterial origin and was not the result of true autohemolysis.

Table 6 demonstrates the effect of incubation on blood at body temperature for 24 hours. The erythrocyte factor is completely destroyed by this treatment. The cells become spherical and there is no rouleau formation.

It has been stated previously that the occurrence of the rouleau phenomenon depends upon the physical and chemical interaction of plasma and erythrocyte factors. It is evident that hemolysis destroys the erythrocyte factor and therefore it must reside in the erythrocyte membrane. The exact structure of this membrane is unknown. However, it is considered to consist chiefly of protein, lecithin, or a related lipid and small amounts of cholesterol.<sup>28</sup> The importance of the lipid content of the erythrocyte was recently demonstrated by Williams et al.<sup>29</sup> There are species differences, and interestingly enough, these differences show a relationship to the species variations in the rouleau-forming tendency.

TABLE 5.—*The Effect of Storage at 5 Degrees C. on the Erythrocyte Factor of Dog Blood*

No. of days stored	One hour sed. rate of washed cells suspended in 1% gelatin in saline
	mm.
1	110
3	64
4	54
17	16

TABLE 6.—*The Effect of Incubation at 37 Degrees C. for 24 Hours on the Sedimentation Rate and on the Erythrocyte Factor*

	Before incubation	After incubation
	mm.	mm.
Blood sedimentation rate.....	50	0
Sedimentation rate of washed erythrocytes in 1% gelatin in 0.85% saline.....	122	1

Brinkman and Wastl<sup>30</sup> attempted to make an ether-soluble concentrate of the saline washings of erythrocytes and demonstrate an effect on the sedimentation rate when this material was added to the blood. This concentrate was found to consist chiefly of lipoids and cholesterol. Kürten<sup>9</sup> had shown previously that lecithin, when added to plasma, delayed sedimentation and cholesterol accelerated it, but, as already stated, his results have been more or less disproved. The effects which he obtained were only slight and are not comparable to the clear-cut effects produced by fibrinogen or gelatin. The results of Brinkman and Wastl were inconclusive. The effects produced by the concentrate were meager. I have been unable to produce a potent concentrate using their method.

It is probable that these lipid substances acting in the plasma can alter the fibrinogen and globulin by adsorption to the molecule and change the interface between plasma and the cell. These experiments do not account for the cellular lipid itself. They merely demonstrate an effect, however slight it may be, produced by these same lipoids when they are present in the plasma.

Since there is a strong tendency for lipoids to form a loose combination with proteins, it is possible that there may be an attraction between the membrane lipid and plasma protein, particularly fibrinogen. Increases in plasma fibrinogen may thus alter the erythrocyte membrane at the interface and change the surface forces so that there is an attraction between the cells produced by dehydrating the surface with the tendency for them to collect into rouleaux.

In order to test this hypothesis, studies of the effect of gelatin on lecithin emulsions and lecithin-coated particles were made. Chemically pure reagents were used and the gelatin was from the same stock used in the previous experiments. This gelatin was found to contain a small amount of calcium and other salts and consequently was not electrolyte free. The concentration of electrolyte was much less than 1 per cent because the ash residue was only 0.99 per cent. Fresh egg lecithin (Merck) (Difco) was used. Lecithin which is more than a year old may not be satisfactory.

The flocculation of lecithin by gelatin apparently has not been described previously. When one prepares an emulsion of 1 per cent lecithin in a 1 per cent gelatin in 0.85 per cent sodium chloride solution and allows it to stand, a flocculation of the lecithin begins in from 3 to 6 minutes and becomes complete in from 15 to 20 minutes. By this time there is a complete separation of the lecithin in the form of a fine curd. The presence of an electrolyte is necessary. The reaction does not occur if dialyzed gelatin and distilled water are used. If undialyzed gelatin and distilled water are used a floc begins to appear after one hour. The amount of electrolyte present in the undialyzed gelatin is apparently sufficient to allow the floc to form after this period of time.

Porges and Neubauer<sup>31</sup> first showed that electrolytes alone will flocculate lecithin emulsions. Since the time of their investigation the phenomenon has been studied several times.<sup>32-27</sup> The concentration of the electrolyte, the nature and charge of the ions, and the hydrogen ion concentration are determining factors in the reaction. Handovsky and Wagner<sup>37</sup> demonstrated that beef serum flocculates lecithin and that it is the globulin and not the albumin fraction which is responsible. They conclude that a lecithin-globulin complex is formed which is unstable in the emulsion and consequently flocculates. Table 7 compares the flocculating power of a pure electrolyte with that of an electrolyte plus gelatin. It is evident that the gelatin greatly augments the lecithin flocculation.

Any explanation of the physical and chemical reactions which take place when gelatin is introduced into a lecithin emulsion will necessarily be hypothetical because our knowledge of the mechanisms of such colloidal behavior is very meager. In the electrolytic flocculation, the reaction between the positive ion and lecithin is perhaps a loose chemical combination. The surface of the lipid particle is changed, the charge is neutralized in part, and the particles failing to repel each other with the original intensity agglutinate without coalescing and form a reversible floc.<sup>35</sup> Perhaps gelatin becomes adsorbed to the lecithin surface in a loose chemical combination, changes the surface of the lipid, and allows the flocculation to take place more readily in a very low concentration of electrolyte.

The importance of electrolytes in the stability of emulsions is spectacularly demonstrated by an experiment first performed by Long.<sup>35</sup> If one prepares an emul-

TABLE 7.—A Comparison between Lecithin-Sodium Chloride and Lecithin-Sodium Chloride-Gelatin Flocculations

Min.	Water	0.01N NaCl	0.1N NaCl	0.2N NaCl	0.3N NaCl	0.4N NaCl
5						
10						
15						
20						
25						
30						
35						+
40						+
45						+
50						+
55						+
60						+
65					+	+
70				+	+	+
75				+	+	+
80				+	+	+
85				+	+	+
90				+	+	++
24 hr.				+	+	++

With gelatin added to 1% concentration

5			+	+	+	++
10			+	+	++	++
15			+	++	++	++
20			+	++	+++	++
25			++	+++	+++	+++
30		+	+++	+++	+++	+++
35		+	+++	+++	+++	+++
40		++	+++	+++	+++	+++
45		++	+++	+++	+++	+++
50	+	++	+++	+++	+++	+++
55	++	++	+++	+++	+++	+++
60	++	++	+++	+++	+++	+++
65	++	++	+++	+++	+++	+++
70	++	++	+++	+++	+++	+++
75	++	++	+++	+++	++++	+++
80	++	++	+++	+++	++++	+++
85	++	++	+++	+++	++++	+++
90	++	++	+++	+++	++++	+++
24 hr.	++	++	+++	+++	++++	+++

sion of lecithin in distilled water and then attempts to extract the lecithin by shaking the emulsion with ether, it is impossible to get the slightest trace of lecithin into the ether. However, when a slight trace of electrolyte is added, the

lecithin is immediately and completely taken up by the ether. The electrolyte ions apparently orient themselves at the lecithin-water interface and change the physical properties of the emulsion.

It is possible to augment the electrolyte flocculation of lecithin by introducing other hydrophylic colloids. Agar and tragacanth are almost as active as gelatin. Gum arabic has no appreciable effect. As mentioned previously, Handovsky and Wagner showed that serum albumin had no flocculating power, whereas whole serum did. This led them to conclude that serum globulin had a strong flocculating effect on lecithin. Egg albumin produces no flocculation.

In order to make the analogy between lecithin flocculation and rouleau formation and blood sedimentation more concrete, it was decided to test the effect of gelatin solutions on inert particles of matter which had been coated with a film of lecithin. Vermilion cinnabar particles were chosen because they approximate the erythrocyte in amplitude and uniformity of size. Their shape, however, is very irregular. Carbon and kaolin particles are too small and spores and starch granules are too large. The cinnabar was lecithinized by saturating 3 grams of it in a mortar with a 1 per cent solution of lecithin in ether. The mass was stirred until a thick paste had formed as the ether evaporated. The powder which remained after the ether had completely evaporated was then used in the experiments.

A suspension of lecithinized cinnabar in 0.85 per cent saline is quite stable, while a suspension of plain cinnabar is most unstable and settles out within a few minutes. The lecithin adsorption to the particle apparently gives it a strong charge and causes the particles to repel each other. If 1 per cent gelatin is added to the lecithinized cinnabar suspension, a slow even flocculation occurs and the mass begins to settle out in a manner similar to the sedimentation of erythrocytes. Figure 1 shows the difference between a saline-lecithin suspension and a saline-lecithin-gelatin suspension of cinnabar particles after standing one-half hour.

The flocculation as seen under the microscope is equally interesting. The lecithinized cinnabar particles in the saline preparation remain isolated as discrete bodies just as washed erythrocytes in a saline suspension. When gelatin is present; however, the particles attempt to line up in a network of chains and appear somewhat like a mycelium. The flocculation is in a linear form just as it is in the rouleau. The microscopic appearance of the floc on cover glass preparations of lecithin-gelatin emulsions also shows the mycelium-like organization of the droplets.

It is evident that there is a certain parallel relationship between rouleau formation and the flocculation of lecithin by certain hydrophilic colloids, and it is possible that the explanation of rouleau formation lies, at least in part, in this relationship. We have seen that the rouleau phenomenon depends upon the interaction of the lipid surface of the cell with a protein solution and that certain proteins which are not native to the blood plasma react with lecithin and lecithin-coated particles just as they do with erythrocytes. The gelatin-lipid surface reaction is therefore most probably responsible for the rouleau phenomenon.

Is the rouleau formation in native blood the result of a similar surface reaction between the lipid cellular membrane and the plasma fibrinogen and globulin?

Certain experiments show that erythrocytes behave similarly to lecithin. Porges and Neubauer<sup>31</sup> demonstrated that lecithin flocculation by electrolytes follows the pattern of Hoffmeister's ionic series. Radsma<sup>39</sup> showed that the agglutination of erythrocytes by a 4.15 per cent glucose solution is hindered by the presence of ions and that the effectiveness of the particular ions was in the same order as Hoffmeister's ionic series. Similarly Höber<sup>10</sup> noticed that hemolysis by hypotonic saline was hindered according to the series. The properties of the erythrocytes therefore are definitely linked with their lecithin content.

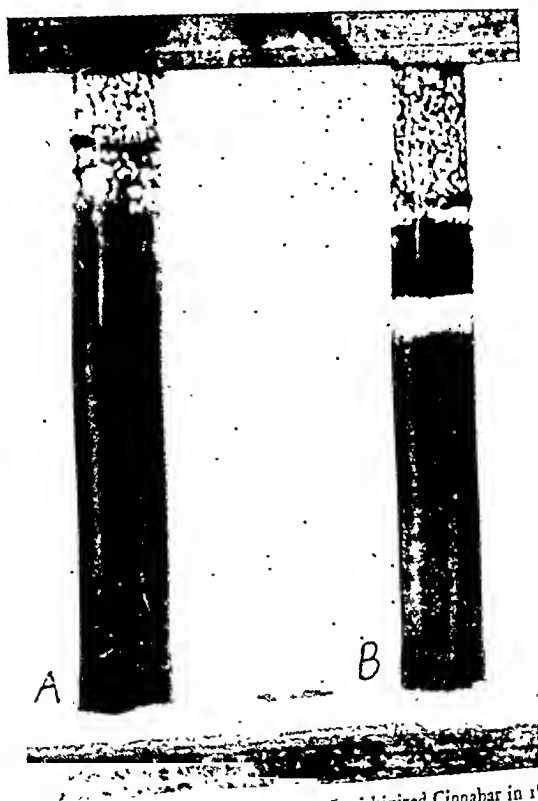


FIG. 1. A. Lecithinized Cinnabar in 0.85% NaCl. B. Lecithinized Cinnabar in 1% Gelatin in 0.85% NaCl. The photograph was taken at the end of a half hour.

After the effect of gelatin on lecithin emulsions was observed it was decided to substitute blood plasma, fibrinogen, globulin, and albumin for gelatin with the hope that fibrinogen and globulin might have an effect similar to gelatin and that plasma from rapidly sedimenting blood either from horse or man would flocculate the suspension. The results were exactly the opposite of what might have been expected (table 8). Horse albumin and pseudoglobulin flocculated lecithin emulsions whereas horse fibrinogen and euglobulin had no effect. Lecithinized cinnabar particles suspended in horse or human plasma with a high sedimentation factor did not settle as in the gelatin solution. I am unable to explain these seemingly

incongruous observations at this time. There is some evidence that gelatin will not flocculate lecithin that has been previously suspended in an albumin solution but will flocculate lecithin that has been previously suspended in a fibrinogen solution.

The naturally occurring erythrocyte lecitho-protein surface is not reproducible in the test tube at this time. Explanations of the cause and mechanics of rouleau formation are therefore only theoretical. It is possible that the normal erythrocyte has a surface of lecitho-globulin or lecitho-fibrinogen. This surface becomes dehydrated in the presence of more than the normal amounts of the homologous protein in the plasma, and rouleau agglutination occurs. The erythrocyte lecitho-protein surface varies pathologically and at times physiologically. Qualitative and quantitative changes in the chemical composition of this lecitho-protein and of the plasma proteins result in more or less rouleau agglutination with a correlative erythrocyte sedimentation rate.

TABLE 8.—*Degree of Flocculation in Lecithin Emulsions in Plasma Protein Solutions at the End of One-Half Hour*

0.85% sodium chloride . . . . .	o
1% gelatin in 0.85% sodium chloride . . . . .	+++
1% egg albumin in 0.85% sodium chloride . . . . .	o
2% horse fibrinogen in 0.85% sodium chloride . . . . .	o
2% horse euglobulin in 0.85% sodium chloride . . . . .	o
2% horse pseudoglobulin in 0.85% sodium chloride . . . . .	+++
2% horse albumin in 0.85% sodium chloride . . . . .	+++

#### SUMMARY

1. The erythrocyte factor in the blood sedimentation phenomenon is not influenced by changes in size or shape of the erythrocytes unless the surface is altered by a hemolytic agent.
2. The erythrocyte factor varies considerably in health and disease. It is depleted when blood is stored under refrigeration.
3. Egg lecithin emulsions and particles of cinnabar covered with lecithin are flocculated by gelatin solutions. The phenomenon is similar to the formation of rouleaux in gelatin solutions.
4. An attempt to explain rouleau formation as a flocculation reaction between plasma proteins and the lecitho-protein surface of the erythrocyte is made.

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# CASE REPORT

## THE CO-EXISTENCE OF CHRONIC LEUKEMIA AND PREGNANCY

By JONAH G. LI, M.D., ALICE MCBRIDE, B.A., AND STACY R. METTIER, M.D.

THERE is no unanimity of opinion among clinicians with regard to the influence of pregnancy on the prognosis of leukemia. Vignes<sup>1</sup> maintains that signs and symptoms of leukemia are temporarily enhanced by pregnancy, but usually improve with parturition. Angelucci<sup>2</sup> and Kasmak<sup>3</sup> are of the opinion that the symptomatology of leukemia becomes more marked with the progression of pregnancy. The latter author advocates the termination of pregnancy or induction of labor when the diagnosis of leukemia is established during the course of cyesis. Grier and Richter<sup>4</sup> also concur that pregnancy may induce a relapse of the leukemic process. On the other hand, Kaplan and Connery,<sup>5</sup> Forkner,<sup>6</sup> McGoldrick and Lapp,<sup>7</sup> Moloney and Heffernan,<sup>8</sup> claim that pregnancy has no influence whatsoever on the prognosis of chronic myelogenous leukemia. The data accumulated by these authors does not bear out the contention that pregnancy rapidly precipitates a fatal outcome for the patient. As a matter of fact, surgical intervention of pregnancy may be detrimental to the life of the mother because of possible precipitation of an acute phase of leukemia, or uncontrollable hemorrhage. Hochman<sup>9</sup> states that a leukemic patient has a good chance of carrying her pregnancy to term without undue harm to herself.

It is the purpose of this paper to report 4 primiparae with chronic myelogenous leukemia who carried their pregnancies uneventfully to term with the delivery of normal nonleukemic children.

### REPORT OF CASES

*Case 1.* Mrs. P. M. H., a 27 year old white housewife, 3 years prior to marriage, had received roentgen irradiation at varying intervals for chronic myelogenous leukemia. After 2 years of married life, the patient became pregnant. When first seen at the age of 23, the symptoms presented were insidious onset of tiredness and general malaise of approximately 2 months duration. There had been slight bleeding of the gums, and her skin bruised easily with slight trauma. There was no definite history of blood dyscrasia in the family, but a sister was thought to be anemic.

*Physical examination on first entry.* The patient appeared pale and asthenic, and showed evidence of recent loss of weight. The skin and mucous membranes were pale, but neither purpura nor petechiae were apparent. Moderately enlarged lymph nodes were felt in the neck, axillae, and groins. The liver edge was felt 2 cm. below the right costal margin. It was sharp and nontender. The spleen was enlarged down to the pelvic brim. There was no edema of the ankles.

*Laboratory data.* The blood examination revealed: hemoglobin, 57 per cent (Sahli); erythrocytes, 2,460,000 cells per cm.; leukocytes, 277,000 cells per cm. of blood. The differential count was: segmented polymorphonuclear leukocytes, 11.0 per cent; nonsegmented leukocytes, 50.0 per cent; small lymphocytes, 3.0 per cent; metamyelocytes, 7.0 per cent; myelocytes, 28.0 per cent; myeloblasts, 1.0 per cent.

*Diagnosis.* Chronic myelogenous leukemia.

From the Division of Medicine, University of California Medical School, San Francisco, Calif.

*Course of illness.* Total body roentgen irradiation was started soon after the diagnosis of chronic myelogenous leukemia was established. Within 1 month following repeated exposures to x-ray irradiation, the white blood count dropped to 11,650 cells per cm.; erythrocytes increased to 3,110,000 cells per cm. of blood, and the hemoglobin increased to 71 per cent (Sahli). During the subsequent  $1\frac{1}{2}$  years, the white blood count seldom exceeded 20,000 cells per cm. of blood, and the hemoglobin remained within a range of 90 per cent (Sahli). The spleen and liver were seldom palpable during this time.

During pregnancy, the patient was examined and the status of her blood evaluated at biweekly intervals. At no time during the entire period of gestation did the white blood count rise above 20,000 cells per cm. of blood, nor did the hemoglobin drop below 90 per cent (Sahli). Only the usually prescribed hygienic measures were observed during the prenatal period. Neither arsenic nor x-ray irradiation was given the patient. At term, a normal female child was born, exhibiting no evidence of leukemia. No complication was encountered during the postpartum period. Six months have passed since the birth of the child. When last examined, the patient appeared in good health and there had been no exacerbation of symptoms. The spleen was nonpalpable, and the blood count was: hemoglobin, 94 per cent (Sahli); erythrocytes 3,870,000 cells per cm.; leukocytes, 19,000 cells per cm. of blood.

*Case 2.* Mrs. D. E. F., a 36 year old white housewife, was admitted November 11, 1943 to the University of California Hospital, complaining of a progressive enlargement of her abdomen and the appearance of hemorrhagic spots over her lower extremities. Ten months prior to this entry, the patient had consulted her physician because of amenorrhoea, and was told that she was 3 months pregnant. Incidental findings at this time were an enlarged spleen and a leukocytosis of 205,000 cells per cm. of blood. No anemia was recorded. The patient was advised to have x-ray irradiation, but she refused to co-operate in carrying out this therapeutic procedure. X-ray irradiation, therefore, was not used in the patient during the period of gestation. The pregnancy progressed without untoward symptoms, and a normal male child, showing no stigmata of leukemia, was delivered at term. The postpartum period was complicated by a slight thrombophlebitis of the right leg which subsided in about a month's time. The white blood cells had remained within a range of 300,000 cells per cm. of blood during the pregnancy. The patient was asymptomatic until three months after parturition when the spleen, which had been previously moderately enlarged, began to increase in size and became markedly tender. There was also a recurrence of the tendency to bruise easily.

*Physical examination at the time of entry.* The patient was thin, pale, and appeared younger than the stated age of 36 years. She seemed to suffer acute pain in the left upper quadrant of the abdomen on sudden motion of the body. Small, discrete, firm lymph nodes were found in the anterior cervical regions, axillae, epitrochlear and inguinal regions. Ecchymoses, about 6 cm. in diameter, appeared over the right knee and over the left thigh. The liver edge was firm, and could be felt about 3 cm. below the right costal margin. The spleen, tremendously enlarged, filled the entire left side of the abdomen and extended into the pelvis, and was markedly tender. No dependent edema of the tissues was apparent.

*Laboratory data.* The blood examination showed: hemoglobin, 49 per cent (Sahli); erythrocytes, 2,740,000 cells per cm.; leukocytes, 478,000 cells per cm. of blood. The differential count was: segmented polymorphonuclear leukocytes, 9.0 per cent; nonsegmented leukocytes, 62.0 per cent; eosinophiles, 1.0 per cent; basophiles, 0.5 per cent; metamyelocytes, 14.0 per cent; myelocytes, 10.5 per cent; promyelocytes, 2.0 per cent; myeloblasts, 1.0 per cent. The platelets were 360,000 per cubic mm. of blood.

*Diagnosis.* Chronic myelogenous leukemia.

*Course of illness.* The patient was treated with radio-active phosphorous, starting with an initial dose of 165 mc. Subsequently she was given varying doses of radio-active phosphorous, 136 to 315 mc. intravenously, at 2 to 8 day intervals. After 8 weeks had elapsed, the hemoglobin was recorded at 77 per cent (Sahli); erythrocytes, 2,630,000 cells per cm.; leukocytes, 282,000 cells per cm. of blood. Then, because of the scarcity of the radio-active salt, total body x-ray irradiation was substituted. In another 4 weeks, the hemoglobin was 90 per cent (Sahli), the erythrocytes count was 4,710,000 cells per cm.; and the white blood count was 18,000 cells per cm. of blood. During this time, there was a gradual reduction in the size of the spleen so that it was felt only with great difficulty. Since then, exposures of the patient to x-ray irradiation have been so spaced as to maintain the level of the circulating white blood cells below 20,000 cells per cm. of blood. The hemoglobin has remained within the range of 90 per cent saturation. The liver and spleen have become vaguely palpable only on occasions.

Three years have passed since the termination of pregnancy. The patient is living and well, and her child appears physically normal.

*Case 3.* Mrs. M. B., a 20 year old white housewife, was first seen July 14, 1938, at the University of California Hospital, with the presenting complaint of insidious weakness, and general malaise over a period of 1½ years. Previous to this hospital entry, the patient had been married for 8 months and had become pregnant shortly thereafter. The period of gestation was uneventful until the seventh month of pregnancy at which time, she had a severe attack of vaginal bleeding which lasted 7 days and necessitated vaginal packing to check the hemorrhage. The patient was well for the next 2 weeks when she went into labor. The pregnancy was only of 7½ months' duration, but the child appeared to be a normal male on examination. No unusual bleeding was encountered at the time of delivery. A routine blood count disclosed: hemoglobin, 63 per cent (Sahli), erythrocytes, 3,170,000 cells per cm., leukocytes, 115,000 cells per cm. of blood. The differential count was: segmented polymorphonuclear leukocytes, 38.5 per cent; nonsegmented leukocytes, 22.5 per cent; small lymphocytes, 5.5 per cent; monocytes, 4.5 per cent; metamyelocytes, 13.5 per cent; myelocytes, 16.5 per cent.

Two weeks after parturition, the patient had another occurrence of severe vaginal hemorrhage which confined her to the hospital for 16 days. When discharged from the hospital, she was gaining in strength, but was conscious of an enlarged mass at the upper left quadrant of her abdomen which was tender upon pressure.

*Laboratory data.* The blood examination disclosed: hemoglobin, 68 per cent (Sahli); erythrocytes, 2,580,000 cells per cm.; leukocytes, 183,000 cells per cm. of blood. The differential count was: segmented polymorphonuclear leukocytes, 14.0 per cent; nonsegmented leukocytes, 21.0 per cent; metamyelocytes, 45.0 per cent; myelocytes, 16.0 per cent; myeloblasts, 4.0 per cent. The platelet count was 346,000 per cm. of blood.

*Diagnosis.* Chronic myelogenous leukemia.

*Course of illness.* The patient was given small doses of radio-active phosphorus (200 to 400 mc.) intravenously at weekly intervals. In 8 weeks' time, the hemoglobin was recorded to be 80 per cent (Sahli), erythrocytes, 4,090,000 cells per cm.; leukocytes, 16,000 cells per cm. of blood. The spleen became gradually and progressively smaller until it was no longer palpable. Her leukocytes were maintained at a level below 20,000 cells per cm. of blood by periodic injections of small doses of radio-active phosphorus. The patient was well for the next 18 months and able to carry on with her housework without difficulties. At the end of this period, the patient had an acute exacerbation of symptoms. Her spleen became progressively enlarged and tender in spite of repeated radio-active irradiation therapy. Roentgen irradiation to the spleen had no effect in reducing its size. A progressive anemia developed but the leukocytes remained at the range of 6,000 to 10,000 cells per cm. of blood at all times. This phase of the disease lasted 2 months and terminated with the death of the patient. Her child remained normal in all respects with no evidence of leukemia.

*Case 4.* Mrs. E. E., a 22 year old white housewife, had consulted her physician a year previously because of amenorrhoea, and was told that she was pregnant. She had been married for 3 years but had had difficulty in conceiving during all this time. A routine physical examination and complete blood examination were found to be within normal limits at that time. The entire period of gestation progressed uneventfully, except for a moderate hypochromic anemia of 60 per cent (Sahli), discovered during the second trimester of the pregnancy. A difficult labor was encountered at term because of foot presentation of the child with persistent arrest of the occiput in the posterior position. The delivery was long and difficult, requiring the use of forceps, and the infant was delivered dead. The postmortem examination disclosed that the child was normal without evidence of leukemia but atelectasis of the lungs was found to be the cause of death. On the second day postpartum, the patient had a rise in temperature to 38° C. A routine blood count disclosed a leukocytosis of 180,000 cells per cm. of blood, and a hemoglobin of 40 per cent (Sahli). A sternal marrow aspiration was done and the marrow was found to be moderately hyperplastic with a preponderance of promyelocytes and myelocytes. It is compatible with the diagnosis of chronic myelogenous leukemia.

*Physical examination.* The patient appeared to be well developed and well nourished. The skin and mucous membranes were pale, but no petechiae or ecchymoses were present. Discrete, small lymph nodes

were found in her cervical region, axillae and groins. The liver edge was felt 6 cm. below her right costal margin. A firm, tender spleen was found to be enlarged down to the level of the umbilicus. There was no dependent edema.

*Laboratory data.* A hematologic examination revealed: hemoglobin, 37 per cent (Sahli); erythrocytes, 1,310,000 cells per cm.; leukocytes, 160,000 cells per cm. of blood. The differential count was: segmented polymorphonuclear leukocytes, 10.0 per cent; nonsegmented leukocytes, 34.0 per cent; monocytes, 3.0 per cent; lymphocytes, 4.0 per cent; eosinophiles, 1.0 per cent; basophiles, 2.0 per cent; metamyelocytes, 21.0 per cent; myelocytes, 23.0 per cent, and myeloblasts, 2.0 per cent.

*Diagnosis.* Chronic myelogenous leukemia.

*Course of illness.* After repeated transfusions, the hemoglobin was noted as 73 per cent (Sahli); and erythrocytes, 3,810,000 cells per cm. of blood. Three weeks after the initial transfusion, the patient became markedly jaundiced with moderate tenderness over the liver. The icterus index was 140 units. Herpes simplex were found on her perioral regions. There was progressive enlargement of her spleen. A course of roentgen irradiation directed over the spleen was started. At the time this paper was being prepared, the patient was still in the hospital but was gaining strength.

#### DISCUSSION

It is unusual for leukemic women to become pregnant. The reason for the infertility of patients has not been established. Perhaps their poor physical health and the amenorrhoea generally associated with the disease are some of the influential factors. In studying postmortem materials, it is not unusual for the reproductive organs to be heavily infiltrated with leukemic cells. The entire ovary and the mucosal surface of the uterus may be destroyed; therefore, ovulation and gestation are almost impossible. This is especially apparent in lymphogenous leukemia.

Although the occurrence of leukemia has been reported in newborn infants,<sup>10</sup> there is no evidence that leukemia is an hereditary disease. Cameron<sup>11</sup> calls attention to the efficacy of the placenta as a barrier in keeping the leukemic cells from entering the fetal circulation. Burchenal<sup>12</sup> has been unable to transmit leukemia from the diseased mouse to its offspring. The fear of the leukemic mother having a leukemic child is no indication for termination of the pregnancy.

The deleterious effects of irradiation on the fetus have been pointed out by Rolleston,<sup>13</sup> Murphy, Shirlock and Doll.<sup>14</sup> Angelucci<sup>2</sup> advocates the use of a solution of potassium arsenite (Fowler's solution) in pregnant leukemic women, resorting to roentgen irradiation over the long bones, spleen and mediastinum only when the former medication is no longer effective. The fetus is not exposed to the irradiation by this means.

#### SUMMARY

The co-existence of pregnancy in 4 patients with chronic myelogenous leukemia has been reported. One patient was known to have had chronic myelogenous leukemia 3 years prior to her pregnancy. The diagnosis of leukemia was made during the course of pregnancy in the remaining 3 patients; 1 in the first trimester, the other 2 in the third trimester. No specific therapy was required in any of the patients during pregnancy. Their children at birth showed no stigmata of leukemia.

Current literature on the subject has been reviewed. The consensus is that pregnancy does not influence the prognosis of chronic myelogenous leukemia. During the period of gestation, the symptoms can be controlled by administration of a

solution of potassium arsenite (Fowler's solution) and irradiation therapy over long bones, spleen and mediastinum without exposing the fetus.

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## EDITORIAL

### NEW FORMS OF "IDIOPATHIC" THROMBOCYTOPENIC PURPURA

THROMBOCYTOPENIA is in general due to one of several mechanisms:

1. A deficiency in the materials necessary for normal platelet production by the marrow megakaryocytes, as in pernicious anemia.

2. A reduction in the actual number of megakaryocytes, and thus in platelets, as in leukemia and aplastic anemia.

3. A reduction in platelet formation by the cytoplasm of the megakaryocytes, as in idiopathic thrombocytopenic purpura and in numerous conditions with splenomegaly, and in certain allergic states.

4. A "sweeping-up" effect, in which although the megakaryocytes are presumably normal both in number and in platelet production the latter are "lost" in the peripheral blood vessels. It is with this type of condition that the two papers of Fitzpatrick et al., and Singer, Bornstein and Wile in this issue are concerned.

In the typical form of idiopathic thrombocytopenic purpura, the megakaryocytes are numerous but greatly deficient in platelet production. We conceive of this disorder as a form of hypersplenism,<sup>1</sup> splenectomy being followed by an extreme increase in platelet production. It seems likely that the spleen is functionally abnormal, producing either an abnormal substance or an excessive amount of a normal inhibitor of platelet production. Other forms of idiopathic thrombocytopenia are beginning to be discriminated. Thus, the type associated with splenomegaly may also be classed as a form of hypersplenism. This may occur in such diverse conditions as rheumatoid arthritis, Boeck's sarcoid, Gaucher's disease and cirrhosis of the liver. The platelet diminution may also be due to an excess of platelet inhibitor released by the enlarged spleen.

S. O. Schwartz<sup>2</sup> has pointed out that an allergic reaction on the part of the marrow may result in megakaryocytic changes indistinguishable from those found in the completely idiopathic type. The allergic reaction is however associated with a marrow eosinophilia, which serves to distinguish it from the nonallergic type. Schwartz contends that those cases showing eosinophilia as a rule recover without splenectomy. Before this concept can be fully accepted, further statistical data are required.

"Thrombotic thrombocytopenic purpura," as described in this issue under different designations, poses a new problem to the clinician. Is the case of thrombocytopenic purpura at hand one of the truly idiopathic type in which the marrow megakaryocytes are functionally inadequate, or is it perhaps the disorder first described by Moschowitz, in which megakaryocytes are normal both in number and in function, but the platelets are being swept into small blood vessels where they cause multiple thromboses? Singer discusses the differential diagnostic features which may be diagnostically helpful in a given case. The condition appears to be more common than was previously considered, although the diagnosis has yet to

be made clinically. The prognosis in the thrombotic type is apparently uniformly bad, whereas in the ordinary idiopathic type it is usually good when splenectomy is performed.

WILLIAM DAMESHEK, M.D.

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# ABSTRACTS

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## HEMATOPOIETIC TISSUES

HISTOLOGIC CHANGES OCCURRING IN THE HEMOPOIETIC ORGANS OF ALBINO RATS AFTER SINGLE INJECTIONS 2-CHLOROETHYL VESICANTS: A QUANTITATIVE STUDY. *J. E. Kimbrell*. From the Anatomical Laboratory, University of Virginia, Charlottesville. *Arch. Path.* 43: 253-295, 1947.

The effects of intravenous injections of sulfur mustard and 3 nitrogen mustard vesicants on the hemato-poietic organs of male albino rats were studied. In general, the results were the production of a lympho-penia in all groups of test animals, a reduction in the weight and amount of the lymphoid tissue, and a hypoplasia and hyperemia of the femoral marrow. Erythropoietic tissue was more resistant than leuko-poietic tissue. Mitoses of myeloid cells were inhibited temporarily. Neutrophils on their way to maturity may have been stimulated to complete their maturation while the more immature ones were inhibited. Megakaryocytes seemed to suffer the least. As a rule, the marrow reacted more slowly to the vesicants than did the lymphoid organs.

O. P. J.

THE ACTION OF RIBONUCLEASE ON FIXED TISSUES. *R. E. Stowell and A. Zorzoli*. From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo. *Stain Technology* 22: 51-62, 1947.

One of the methods used for localizing nucleoproteins within cells is to study the stainability following the specific action of the enzyme. Ribonuclease has been used by many investigators and in about as many different ways. Stowell and Zorzoli have attempted to establish the optimal conditions for the histochemical use of this enzyme. The best general fixative was found to be neutral formalin (4 per cent formaldehyde). Of the five buffers tested, McIlvaine's citric-acid-disodium-phosphate buffer at pH 7.0 was the most satisfactory. The length of incubation seemed to have a direct relationship to enzymatic action but, although the action was less active at room temperature, there was little difference between 60°, 50° and 40° C. for effective distinction of cytoplasmic staining. Even a ribonuclease concentration of 0.001 mg./ml. reduced staining considerably after prolonged incubation.

O. P. J.

MORPHOGENESIS, CRENATION AND CYTOLYTIC REACTIONS OF THE ERYTHROCYTES OF AMPHIBIANS. *J. Holtfreter*. From Departments of Zoology, McGill University and University of Rochester. *J. Morph.* 80: 345-367, 1947.

Studies of isolated embryonic amphibian cells have shown them to have 4 protoplasmic layers. These are the plasmasol core containing the nucleus, which is surrounded by a viscid capsule of plasmagel separated from the membrane by a shell of ectoplasmic fluid. The amphibian erythrocyte originates from a spherical amoeboid cell which has these same cytoplasmic layers. The isolated amoeboid erythroblast changes its shape several times before it reaches the final form. The first noticeable change is the "thorn-apple" or crenated form. Later it becomes endowed with a monaxial polarity in which it is elongated with one smooth and one corrugated side. There is considerable evidence to indicate that the outer membrane is the most important formgiving element of the cell. When freshly differentiated erythrocytes are

placed in hypotonic salt solutions they may return to their previous crenated form and even become amoeboid.

O. P. J.

THE FEMORAL BONE MARROW CELLS OF THE ALBINO RAT. *M. Vogel*. From Department of Medicine, New York Medical College. *Am. J. Med. Sc.* 213: 456-462, 1947.

A new technic for obtaining rat femoral bone marrow has been proposed. The essential steps are the dissection of the femur free from the soft tissues and the subsequent removal of marrow by means of a 20 gage syringe inserted into the bisected bone. The marrow is mixed in a drop of saline and then smeared. According to Vogel, the advantage of this method over others is that it permits the counting of consecutive cells rather than those in selected fields. Some years ago, Isaacs (*Science* 68: 547, 1928) developed a somewhat similar method for human sternal marrow. Instead of saline, he used serum, plasma or acetate fluid. It must be realized that in all of these methods, to obtain the best morphologic detail the preparation must be dried rapidly and that this may be prevented by having too much diluting fluid.

O. P. J.

## IRON POISONING

POISONING WITH A PREPARATION OF IRON, COPPER, AND MANGANESE. *G. Forbes*. From the Department of Forensic Medicine, University of Sheffield, Sheffield, England. *Brit. Med. J.* 1: 367-370, 1947.

This report of 2 fatal cases of poisoning from ingestion of a large number of medicinal tablets containing ferrous sulphate, copper sulphate, and manganous sulphate is important in view of the widespread belief that iron salts are harmless when ingested, and because of the current trend of clinical use of medicinal iron preparations containing copper and other metal salts.

Forbes reviews the literature dealing with the toxic effects of iron salts and emphasizes the fact that although reported instances of poisoning from this cause have been extremely rare, they have occurred. He also summarizes the observations relating to toxicity of copper and manganese salts, which are quite definitely toxic when ingested in moderate amounts.

The 2 fatal cases which he reports were children aged 3 years and 1 year respectively. The older child ingested 50 tablets, each of which contained ferrous sulphate *exsic.* 3 gr., copper sulphate 1/25 gr. and manganese sulphate 1/25 gr. The younger child ingested 30 of these tablets. Symptoms were primarily those of profound gastrointestinal irritation and vascular collapse. Death occurred in each instance and autopsy revealed necrosis in the stomach and toxic changes in the liver.

Animal experiments indicated that death could be produced in guinea pigs and cats by administration of the same tablets, and that the large amount of iron was the toxic agent.

J. F. R.

TWO CASES OF FERROUS SULPHATE POISONING. *J. Thomson*. From the Royal Infirmary, Dundee, Scotland. *Brit. Med. J.* 1: 640-641, 1947.

This report re-emphasizes the hazard of ingestion of iron salts by children. One child, 16 months old, died following ingestion of 40 tablets similar to those described in the previous report by Forbes, and a second child aged 2 years was extremely ill following ingestion of 10 such tablets. The symptoms in each case were those of extreme gastric irritation and hemorrhage. The fatal case was autopsied and showed no changes except necrosis of the gastric mucosa.

J. F. R.

## LEUCOCYTIC DISEASES

THE ETIOLOGY OF CHLOROMA AND THE NATURE OF THE GREEN PIGMENT. *J. G. Humble*. From the John Burford Carlill Laboratories, Westminster Hospital, London, England. *Quart. J. Med.* 15: 297-312, 1946.

Three cases of leukemia are presented in which chloroma was found in various locations at post mortem examination. Chronic myelogenous leukemia and acute myelogenous leukemia and monocytic leu-

mia were the 3 types represented. An attempt was made to define the nature of the pigment characteristic of the tumor. A small quantity of green pigment was separated which became bright green in reduction with sodium hydrosulphite and showed green fluorescence with ultra violet light. Evidence is presented from spectroscopic examination to show that the pigment resembles the reduced denatured green pigment globin cholehaemochromogen described by Lembert, Legge and Lockwood (*Biochemical Journal*, 1941). The possible biological mechanisms present in myelogenous and monocytic leukemia which may be responsible for the production of this pigment are discussed.

R. S. E.

**INFECTIOUS MONONUCLEOSIS—COMPLICATIONS.** *F. S. Benn.* From the Department of Medicine, Faculty of Medicine, University of Western Ontario, and the Department of Medicine, Victoria Hospital, London, Ontario, Canada. *M. A. J.* 56: 499-502, 1947.

In this brief review reference is made to cardiac, pulmonary, renal, hepatic, splenic and neurological complications of infectious mononucleosis. Two cases are reported in which death occurred following spontaneous rupture of the spleen. Autopsy in both cases revealed that there had been subcapsular hemorrhage, nearly complete separation of the capsule from the pulp and eventual rupture of the capsule with massive intraperitoneal hemorrhage. The principal microscopic findings at autopsy were prominent accumulations of mononuclear cells in the liver, spleen, lymph nodes and kidneys and small groups of cells in the heart muscle. Nothing of note was found in the bone marrow in either case.

L. E. Y.

**DIABETES MELLITUS, FIBROCONGESTIVE SPLENOMEGALY (BANTI'S SYNDROME) AND INFECTIOUS MONONUCLEOSIS.** *L. L. Pennock and L. E. Lieder.* *Am. J. Digest. Dis. & Nutrition.* 14: 135-142, 1947.

The sole purpose of this paper is to report the concurrence of "Banti's syndrome" and diabetes mellitus, which is apparently unique in the literature. The case is that of a 20 year old American man who was found to have splenomegaly, petechiae, and ecchymoses during investigation of an unrelated upper respiratory infection. Laboratory studies disclosed diabetes mellitus and pancytopenia with a hemolytic component. A bone marrow puncture was considered normal. The spleen was removed; it weighed 800 Gr. and was found to show atrophic follicles, wide sinuses, and fibrosis ("fibrocongestive splenomegaly"). Following operation there was a reduction in the requirement for insulin. As an additional complicating feature, the patient developed infectious mononucleosis a week after operation, with subsequent remission, and a return to normality.

This is an interesting coincidence of two apparently unrelated disorders. The etiology of the splenomegaly remained obscure, as it frequently does in cases of "congestive splenomegaly."

S. E.

**AGRANULOCYTOSIS CAUSED BY THIOURACIL. A REVIEW OF FIFTY-NINE CASES IN THE LITERATURE AND A REPORT OF TWO ADDITIONAL CASES.** *J. H. Morton.* From the Department of Medicine, New York Medical College, New York City. *Am. J. Med.* 2: 53-64, 1947.

The hazards of thiouracil treatment are emphasized and a detailed summary of 59 reported cases of agranulocytosis resulting from medication with this drug is presented. Treatment should consist in omission of thiouracil and administration of penicillin. The author also advises pyridoxine, although evidence for its effectiveness is not completely convincing.

J. F. R.

**GRANULOCYTOPENIA DUE PROBABLY TO "PYRIDENZAMINE."** *IV. B. Blanton and M. E. B. Owens, Jr.* From the Allergy Service, Medical College of Virginia. *J. A. M. A.* 134: 454-5, 1947.

Pyribenzamine is a pyridyl-benzol compound which has been increasingly employed in recent months for its anti-allergic action in hay fever, asthma, urticaria, and similar disorders. Reactions to pyribenzamine are unusual. The present paper reports the development of granulocytopenia ("agranulocytosis") during a course of treatment with this drug, and apparently due to the drug itself.

The patient, a 73 year old woman with a chronic urticarial eruption of obscure etiology, was given some 150 mg. of pyribenzamine daily with marked symptomatic relief. After 8 weeks of such treatment



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# NEWS AND VIEWS

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The following letter has been received from Roger C. Crafts, Ph.D., Department of Anatomy, Boston University School of Medicine, Boston:

To the Editor:

In the March 1947 issue of *Blood*, Doctors E. S. Jones, K. B. McCall, C. A. Elvehjem, and P. F. Clark published a paper entitled "The effect of diet on the hemoglobin, erythrocyte, and leukocyte content of the blood of the rhesus monkey (*Macaca Mulatta*)."<sup>1</sup> I would like to offer a few comments on the findings concerning the neutrophil-lymphocyte ratio only.

These authors reported that the neutrophil-lymphocyte ratio could be reversed from  $\frac{3}{6}$  to  $\frac{6}{3}$  with vitamin B or whole liver factor deficiencies (see fig. 2) and that a "normal" ratio of about  $\frac{3}{6}$  could be produced with a diet "adequately supplemented with all the vitamins and a supply of whole liver substance." Are these changes actually due to diet?

In a paper published in *Endocrinology* in 1941,<sup>1</sup> the data obtained from a 3 month study of 3 normal adult female rhesus monkeys were published. These monkeys exhibited initial total white cell counts of 15.0, 26.5, and 34.6 thousand cells per cu. mm. Each of these monkeys showed a predominance of lymphocytes when differential counts were made, i.e., 67.5, 67.0, and 92.0 per cent respectively. Because of the range in the total white cell counts and the unexpected predominance of lymphocytes, these animals were further studied to determine the actual normal count. Two of the monkeys showed a gradual decrease in total white counts to between 10-15 thousand cells and remained at that level. The third monkey, a particularly fierce animal, showed a decrease from 34.6 to 25.0 thousand cells in 60 days. Because the animal showed no signs of becoming tame, further work on this animal was given up as hopeless. During the 3 month training period the neutrophil-lymphocyte ratio of the first 2 monkeys reversed to a ratio typical of the human. In other words, the neutrophil-lymphocyte ratio reversed with no treatment whatsoever. This reverse is exactly the same as reported by Jones, et al. as due to dietary deficiencies.

As both F. P. Rous<sup>2</sup> and B. F. Davis and A. S. Carlson<sup>3</sup> reported a tremendous pouring of lymphocytes into the blood stream as a result of excitement and muscular exercise, the high white counts and the high lymphocyte level in these monkeys were attributed to the resistance these animals exhibited when being handled. The reverse of the neutrophil-lymphocyte ratio and the decrease in total white cell count mentioned above seemingly were due to the training of the animal to the routine of blood removal during a period of 3 months.

Is this reverse of the neutrophil-lymphocyte ratio due to inadequate diet or to the monkey becoming accustomed to being handled? The diet of the two monkeys mentioned above, to my knowledge, was not changed during the 3 months of observation. Jones, et al. mentioned in their discussion that many of their monkeys, due to excitement, exhibited a high total white cell count when first handled, but do not mention this excitement as a cause for the large number of circulating lymphocytes. These authors also tabulate the blood picture of 12 monkeys from a zoo. Four of these monkeys show the neutrophils predominating while 8 show the lymphocytes in great numbers. If these animals had the same diet, why the discrepancy?

I do not state that Doctors Jones, McCall, Elvehjem, and Clark are in error. I simply raise the question as to the actual cause of the neutrophil-lymphocyte reverse during an experiment on the rhesus monkey

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<sup>1</sup> R. C. CRAFTS: The effect of endocrines on the formed elements of the blood. Part 2: The effect of estrogens on the dog and monkey. *Endocrinology* 29: 606, 1941.

<sup>2</sup> J. Exper. Med. 10: 537, 1908.

<sup>3</sup> Am. J. Physiol. 25: 173, 1909.

and to what the normal differential white cell count of the resting monkey actually is. This is an interesting and important problem and the evaluation of further hematologic work on the monkey would seem to depend on a solution of it.

Roger C. Crafts

Dr. C.A. Elvehjem, Department of Biochemistry, University of Wisconsin, Madison, replies as follows:

To the Editor:

We have read the preceding letter by Dr. Roger C. Crafts with much interest and agree that studies on the actual causes of the observed changes in the neutrophil-lymphocyte ratio in the blood of monkeys is an interesting and important hematologic problem. The possible relationship of endocrines to the production of formed elements of the blood should be given consideration. However, we believe that our studies have been controlled sufficiently to demonstrate that diet does have a specific effect on the neutrophil-lymphocyte ratio in the blood of monkeys.

We have used a large number of animals rather than 2 or 3 as indicated in Dr. Crafts' letter and have carried the animals over sufficient periods of time to eliminate the possible effect of handling. He states that the neutrophil-lymphocyte ratio was reversed in two of his animals with no treatment whatsoever. He does not give the diet used and if his diet were incomplete, such a reversal would be exactly what one would expect when the animals were kept on the diet for several months. We know from experience that different monkeys develop a deficiency in varying periods of time and this would explain why 4 of the animals which we kept at the zoo showed a reversal while 8 of the monkeys did not. The diet which the animals received at the zoo probably did not supply adequate amounts of the monkey anti-anemia factor and during the period at the zoo 4 of the animals developed a deficiency.

The best evidence for the nutritional effect is based on the fact that specific reversals have been obtained in a large number of animals and these reversals have been corrected upon administration of liver and milk. The early observations have been reported by J. M. Cooperman, H. A. Waisman, K. B. McCall, and C. A. Elvehjem<sup>4</sup> and specific effects of milk have been described by J. M. Cooperman, W. R. Rueggier and C. A. Elvehjem.<sup>5</sup> Therefore, under controlled conditions a syndrome has been produced on 2 different diets and corrected by the addition of 2 different food materials. I doubt that better evidence can be presented for the nutritional effect although it is entirely possible that the mechanism of the nutritional effect may be related to the endocrines.

C. A. Elvehjem, M.D.

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The following letter is from Dr. Edwin E. Osgood, University of Oregon Medical School, Portland, to Dr. A. S. Giordano, Secretary-Treasurer, American Society of Clinical Pathologists, 531 North Main Street, South Bend, Indiana:

Dear Doctor Giordano:

The purpose of this letter is to offer suggestions aimed toward clarifying the present confusion in the terms used for the cells and diseases of the blood and blood-forming organs. This confusion is so great at present that it is difficult to interpret a differential cell count on blood or bone marrow from one laboratory or even from different technicians of the same laboratory without personally visiting the laboratory and learning of the criteria of cell identification and disease classification in use there.

Not only have many different names been used for the same cell and disease, but the same names have been used for entirely different cells and diseases. As an example, one cell of the granulocyte series (cell No. 76 in Osgood and Ashworth's Atlas) if observed in different laboratories would be called a neutrophil

<sup>4</sup> J. Nutrition 30: 45, 1945.

<sup>5</sup> Proc. Soc. Exp. Biol. & Med. 62: 101, 1946.

staff cell, neutrophil rod cell, neutrophil stabkernige, neutrophil band cell, neutrophil stab cell, non-segmented neutrophil, nonfilamented neutrophil, neutrophil class I, neutrophil juvenile, neutrophil metamyelocyte, polymorphonuclear neutrophil, or neutrophil rhabdocyte, while the terms metamyelocyte (metagranulocyte) and polymorphonuclear as used elsewhere would not even include this cell. As an example of the confusion in the terminology of disease, the same patient with leukemia might be classified in different clinics as having subacute leukopenic myelogenous leukemia, acute aleukemic myeloid leukemia, stem cell leukemia, myeloblastic leukemia, subacute aleukemic myelocytic leukemia, acute myelosis, acute leukemia, subacute subleukemic granulocytic leukemia, or misclassified as acute lymphoid, lymphogenous or lymphocytic leukemia. The confusion is not in the cell or disease. All or nearly all the different observers would have a clear idea of the proper place of the cell and probable course of the disease. While most of the terms are probably correctly interpreted by hematologists who are familiar with the publications of the person using the term and who may have visited his laboratory or clinic and seen his particular criteria of cell identification and disease classification, the average general practitioner, clinical pathologist, or even internist cannot be expected to have this degree of familiarity with the criteria used elsewhere and often is unfamiliar with the criteria used in the various laboratories and clinics even in his own city. The basis for the confusion appears to be that terminology has gradually developed with each author suggesting his own terms and many failing to clearly illustrate and describe sharp lines of demarcation that are easily recognized by others.

An attempt was made by the writer to solve the problem by publishing in the "Atlas of Hematology" by Osgood and Ashworth tables of cell nomenclature and identification and illustrations of both typical, atypical and borderline cells of each series with the criteria of identification and differentiation from other cells beside them, giving clear-cut criteria for the borderline cells and tables of criteria for differentiation of diseases of the blood and blood-forming organs. Some of these tables and criteria were also published in Osgood's "Textbook of Laboratory Diagnosis," third edition. This attempt has been a failure as indicated by the infrequent use of these terms outside of the Northwest and the fact that these terms have not been adopted by any major publication or clinical group. When the attempt was made, it was made with the one objective of clarifying a confused subject. The writer holds no brief for the terms he suggested but does feel that the time is ripe for clarification by a competent committee.

I suggest that a committee be appointed to meet during the week before the meeting of the American Society of Clinical Pathologists in Chicago, October 28-30, 1947, to make recommendations to that body as to the preferred name and the criteria of differentiation for the cells and diseases of the blood and blood-forming organs. It is suggested that the membership of the committee include representatives from the group of clinical hematologists known as the Hematology Club, the International Society of Hematology, the American Society of Clinical Pathologists, interested groups in Canada and Great Britain, and at least one member or consultant who is a lexicographer well versed in Latin and Greek. Recommendations should include specific criteria for distinction between borderline cells and diseases and include a corresponding classification for both the Romanovsky and supravital techniques. Later or in conjunction with this meeting, a similar committee could meet for each country or group of countries constituting one language group to determine the comparable preferred names in that language.

I would further suggest that, as a guide for this committee and to provide the committee with the opinions of all interested persons, a copy of this letter be published in the correspondence section of the *Journal of the American Medical Association*, the *Journal of Laboratory and Clinical Medicine*, *Blood*, *The Journal of Hematology*, and the *American Journal of Clinical Pathology*, and that in the latter two journals there be included tear-out pages or folded inserts similar to that illustrated below for recording the usage and preference of all interested persons. This data should give the committee a clear conception of the degree of present confusion and also of the preferences of those who are actually using the terms. The recommendations of the committee could then be presented to the American Society of Clinical Pathologists at its fall meeting for acceptance or rejection and an approved plan could be presented to the other interested groups at their next meetings. They would still stand merely as recommendations, there being nothing compulsory about the use of the suggested terms, but they might serve as a guide to editors, authors and clinicians and directors of clinical laboratories and in time might become generally accepted terms, which would greatly simplify the task of reading hematologic literature with understanding and full comprehension of the meaning of the author and lead to greater accuracy in the interpretation of data to day laboratory reports.

The major objection which might be raised is that standardization tends to halt progress. That is

true, and it is certain that the terms should be revised by committee action every 5 or 10 years and that new terms may need to be added and old ones deleted from time to time. It is equally certain that not all persons will at first accept the recommendations of the committee, but it seems worthwhile to see if there is not now a sufficient area of agreement to permit a real clarification in the criteria for cell identification and disease classification in the field of hematology as has been done by the American Heart Association for cardiovascular disease and the American Rheumatism Association for arthritis. Then the disease terminology recommended could be incorporated in the next edition of the *Standard Classified Nomenclature of Disease*.

Copies of this letter are being sent to Stanley P. Reimann, President of the American Society of Clinical Pathologists, Joseph M. Hill of the International Society of Hematology, William Dameshek, Editor of *Blood, The Journal of Hematology*, and a representative member of the informal Hematology Club formed at the last meeting of the American Society of Clinical Investigation, Morris Fishbein, Editor of the A. M. A. publications, S. E. Gould, Editor of the *American Journal of Clinical Pathology*, Carl Moore, Editor of the *Journal of Laboratory and Clinical Medicine*, H. E. MacDermot, Editor of the *Canadian Medical Association Journal*, and T. F. Fox, Editor of *Lancet*.

Very truly yours,  
Edwin E. Osgood, M.D.

Condensed example of blank to be used for the information of the committee appointed to make recommendations as to nomenclature of cells and diseases of blood and blood-forming organs.

If you are in charge of a clinical laboratory or an internist interested in clinical hematology, you are requested to fill out, sign, and mail before October 1, 1947, the blanks below to A. S. Giordano, Secretary-Treasurer, American Society of Clinical Pathologists, 531 North Main Street, South Bend, Indiana.

The numbers refer to the cells in Osgood-Ashworth, "Atlas of Hematology," but if you prefer, numbers of cells in any other standard atlas or journal may be substituted if the reference is given and blanks are provided for inclusion of other cell types.

<u>Cell No.*</u>	<u>Name now used</u>	<u>Name preferred if generally agreed upon</u>
1. _____		
2. _____		
3. _____		
4. _____		
5. _____		
• _____		
• _____		
316. _____		

\* Use these numbers if cells referred to are those illustrated in Osgood and Ashworth "Atlas of Hematology." Insert number in blank if other reference is used and give reference.

#### For Diseases

Underline name now used and circle name preferred in following list. Note blank for adding name not listed.

(Example)

1. Hemolytic anemia of newborn; erythroblastosis fetalis; icterus gravis neonatorum; hydrops fetalis; congenital anemia; erythroleukoblastosis; \_\_\_\_\_

2. \_\_\_\_\_

3. \_\_\_\_\_

• \_\_\_\_\_

• \_\_\_\_\_

Attach any comments on separate sheet. If you do not refer to published illustrations and description, be sure to include exact criteria of differentiation from other cells or diseases with which it might be confused.

Under the sponsorship of Dr. Alexander S. Wiener of Brooklyn, New York, a meeting was held in Atlantic City on June 8, 1947 for the purpose of forming an American Society for the Study of the Blood. About 25 physicians attended. After much discussion it was decided by those present to support an informal Hematology Club (*Blood*, 2: 404, 1947) to meet just prior to the annual May meetings of the Society for Clinical Investigation and the Association of American Physicians. It was felt that this Hematology Club which was sponsored by internists with relatively broad interests, was to be preferred to an organization comprising a rather narrow group of specialists having transfusions and blood groups as their chief interest. It was conceivable that from the former group might evolve at some later date a full-fledged national organization. It was also the consensus that full support should be given to the newly formed International Society of Hematology, which had much to offer in fostering international relationships and which would hold biennial meetings in different countries. Motions by Dr. Wiener to form, (1) a new American Society for the Study of the Blood, and (2) an American Society for the Study of Blood Groups and Transfusions were defeated.

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Joseph M. Hill, director of the William Buchanan Blood Center, Baylor University, Dallas, Texas, and professor of clinical pathology at Southwestern Medical College, received the first Marchman Award for notable research in medicine at the annual dinner of the Dallas Southern Clinical Society, January 21. Dr. Hill won the award for his recent investigations of the Rh factor.

Dr. Cecil J. Watson of Minneapolis has been elected recorder of The Association of American Physicians at their 60th annual meeting in Atlantic City on May 7th.

The Swiss Hematologic Society was founded on November 17, 1946. Professor Alder of Aarau was elected the first president. The Society was founded as one of the subsections of the Swiss Society for Internal Medicine.

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As we wish the 1947 Index of *Blood* to include the Special Rh Issue (immediately following the present issue), the Index will appear in February 1948 instead of the current issue, as previously planned.

#### Errata

The following information has been received from Dr. Solomon Estren. On page 92, volume II, *Blood* (January 1947) in the article, "Congenital hypoplastic anemia associated with multiple developmental defects (Fanconi syndrome)," by Estren, Suess, and Dameshek, the second paragraph on the page concludes with the statement: "Death here also occurred three years later." This should have read: "This patient is living and well seven years after the operation (personal communication, Dr. J. V. Dacie<sup>5</sup>)."

A communication from Dr. J. H. Whitlock (The use of photo-electric turbidometry in the determination of red cell counts, hematocrits, and hemoglobin. *Blood* 2: 463, September 1947) states that he has the D.V.M. and M.S., but not the Ph.D. as used erroneously after his name on the title page of the article.

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# BOOK REVIEW

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*Die Milzpunktion* By SVEN MOESCHLIN (Foreword by Professor W. Löffler). Benno Schwabe & Co., Verlag, Basel (Switzerland). Pp. 205, 1947. 30 francs.

This is the first monograph devoted entirely to splenic puncture and represents a description of the author's experience with this technic in 180 cases observed at the University Clinic at Zurich during the seven year period between 1939-1945. The book is beautifully printed on excellent paper and contains many photomicrographs as well as two plates in full color.

The book deals largely with the technic, indications and results of splenic puncture in hematologic cases, particularly those with splenomegaly. The author points out in his introduction that puncture should be undertaken only in well-defined splenomegaly and is contraindicated in the presence of a hemorrhagic disorder and in septic splenomegaly as well as in cases with painful spleens. A fine sutured needle is introduced in the ninth or tenth interspace over the point of maximum splenic dullness during inspiration and a small amount of material aspirated. This is then smeared and stained. Excellent descriptions of the normal cellular morphology are presented, then followed by minor variations, and finally by the findings in various disease conditions. The diagnostic value of the splenogram in certain hematologic problems is presented in a series of cases.

The author concludes that the method is without danger in properly selected cases. In the splenomegaly of cirrhosis of the liver and splenic vein thrombosis, the splenogram is practically normal, and only minor deviations from the normal are present in hemolytic anemia. Septic splenomegaly is easily differentiated from leukemia. In infectious mononucleosis, there is a great overactivity of the reticulo-endothelial and lymphoid cells. In Hodgkin's disease the puncture has particular diagnostic merit, especially in those cases in which a lymph node is not available for biopsy. Chronic myelogenous leukemia was extensively studied both before and after treatment with x-rays, arsenic and urethane. Particular attention was paid to mitotic figures, which appear to be selectively affected by urethane.

There can be little doubt that splenic puncture, as practised by the author, is of distinct value, especially as an adjuvant to other hematologic studies, more particularly the blood and the marrow puncture. Although splenic puncture is not diagnostically essential in the great majority of "blood" cases it will occasionally yield valuable information and to the conscientious investigator will give a background of useful knowledge which is of distinct help in understanding the physiologic pathology of certain disease processes.

Sternal puncture has yielded invaluable information regarding what goes on "behind" the blood picture. It is not inconceivable that splenic puncture may point to features of importance in understanding the mechanisms of the hypersplenic states including splenic neutropenia, thrombocytopenia, anemia and pancytopenia. Although the author barely touches upon these subjects in this monograph, he has performed a highly creditable piece of work in documenting his experiences so well. An excellent bibliography and a good index complete the book.

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## HEMOPOIESIS IN RIBOFLAVIN-DEFICIENT RATS

By K. M. ENDICOTT, M.D., ARTHUR KORNBERG, M.D., AND  
MAURINE OTT, A.B.

IN THIS laboratory, experimental granulocytopenia and anemia in rats have been produced by a number of dietary deficiencies, including "folic acid" (pteroyl-glutamic acid) deficiency,<sup>1, 2</sup> riboflavin deficiency,<sup>3</sup> pantothenic acid deficiency,<sup>4</sup> and deficiency of essential amino acids.<sup>5</sup> A detailed study of the hemopoietic tissues in folic acid deficiency and their response to folic acid therapy has been reported.<sup>1</sup> The present study is a comparison of hemopoietic tissues in the granulocytopenia and anemia of riboflavin deficiency with the hemopoietic tissues in folic acid deficiency, including the response of these tissues to vitamin therapy.

In the studies of Kornberg, Daft, and Sebrell,<sup>3</sup> rats fed a diet deficient in riboflavin developed granulocytopenia in about 50 per cent of the cases and, less frequently, anemia. The granulocytopenia responded to treatment with folic acid in 30 or 33 rats but responded to riboflavin therapy in only 6 of 28 rats. Riboflavin alleviated the anemia in 10 of 17 rats while folic acid failed to do so in all of 7 rats. In the present study, therefore, rats having granulocytopenia were treated with folic acid while rats having both granulocytopenia and anemia were treated with riboflavin.

The experimental conditions with few exceptions have been similar to those of the previous study of hemopoiesis in folic acid deficiency<sup>1</sup> so as to permit accurate comparison of hemopoiesis in the two deficiencies. Both studies were carried out by a quantitative method in which an index of the total quantity of each type of cell in the bone marrow is obtained. This is accomplished by determining the average cellularity of the marrow and the differential count of marrow cells. The average per cent of cellularity is multiplied by the per cent in the differential to arrive at a quantitative index of the total amount of each type of marrow cell.

### EXPERIMENTAL METHODS

At weaning, albino rats of both sexes of Osborne and Mendel strain were fed purified diet number 950<sup>3</sup> ad libitum. This consisted of leached, alcohol-extracted casein (18 per cent), dextrose (68.76 per cent), hydrogenated vegetable oil (8 per cent), salt mixture (4 per cent),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.08 per cent), and ferric citrate (1.16 per cent). To each 100 grams of this diet were added thiamine hydrochloride (1 mg.), pyridoxine hydrochloride (1 mg.), calcium pantothenate (4 mg.), nicotinamide (2 mg.), 2-methyl-1,4-naphthoquinone (vitamin K) diacetate (0.4 mg.), biotin (0.001 mg.), and choline chloride (200 mg.) dissolved in 1.0 cc. of 20 per cent ethanol. Twice weekly each rat received 0.25 cc. of corn oil containing 2500 units of vitamin A and 200 units of vitamin D, and once weekly 3 mg. of  $\alpha$ -tocopherol in 0.03 cc. of ethyl laurate. The study extended over a period of 15 months.

From the Pathology Laboratory (K. M. Endicott and Maurine Ott) and the Division of Physiology (Arthur Kornberg), National Institute of Health.

Eight rats from two or more litters were placed on the experimental regimen each week. Within the first week the following determinations were carried out on each rat using freely flowing tail blood: hemoglobin (Evelyn photoelectric colorimeter), hematocrit (van Allen), erythrocyte count (in duplicate, diluting in Trenner pipets), leukocyte count (in duplicate, Trenner pipets), differential leukocyte count (200 cells), and reticulocyte count (with the Miller disk\*).

The rats were observed daily and the leukocyte and differential counts and the hematocrit determinations were repeated when any of the following signs were noted: facial porphyrin stains, pallor, loss of hair, dermatitis, general weakness, and loss of weight. When these check counts indicated that the rats were suitable for special hematologic study, complete blood determinations, as above, were repeated and the rat was studied under one of the following experiments:

(a) In order to study the hemopoietic tissues of rats with severe granulocytopenia, 12 rats were killed when their polymorphonuclear counts fell below 150 per cubic millimeter.

(b) A corresponding group of 21 rats with similar or more severe granulocytopenia were given daily oral doses of 25 micrograms of crystalline folic acid† for periods of 4 days (8 rats), 8 days (6 rats), and 12 days (7 rats). In each case complete blood determinations were carried out at the end of the treatment period and the rats were killed for detailed studies of hemopoietic tissues.

(c) Another series of 11 rats with both granulocytopenia and anemia (hematocrit below 30 vol. per cent) were studied without treatment for comparison with the series (a) rats showing granulocytopenia alone.

(d) A group of 4 rats with hematocrit values below 30 vol. per cent were treated for 10 days with daily oral doses of 100 micrograms of riboflavin and were killed and examined for the purpose of noting the effect of this therapy on the hemopoietic tissues.

(e) For the purpose of studying the evolution of the dyscrasias rats were selected according to the following criteria: (1) The blood picture must have been normal at the beginning of the experiment. (2) A period of at least 1 month on diet number 990 must have elapsed. (3) A steady downward trend of the granulocyte count must have been shown. (4) There must have been no anemia at any time. Thus this series comprised 7 rats with polymorphonuclear counts over 1000 per cubic millimeter, 4 rats with counts between 500 and 1000, 5 rats with counts between 150 and 500, and 12 rats with counts of less than 150 polymorphonuclears per cubic millimeter.

In all experiments the blood of each rat was examined on the day of death. Rats were killed with illuminating (mixed natural and coal) gas. Giemsa-stained smears of marrow from the left femur were prepared by the plasma diluting method,<sup>7</sup>

\* Microscope ocular disk designed by Dr. John W. Miller, manufactured by Bausch and Lomb Optical Company. The disk is ruled with one square ( $7 \times 7$  mm.) containing a smaller square in one corner having one ninth the area of the larger square. For each of 50 fields the erythrocytes in the smaller square and the reticulocytes in the larger square are counted.  $\text{Reticulocytes} \div 9 \times \text{erythrocytes} = \text{reticulocytes}$ .

† Furnished by courtesy of Dr. E. L. R. Stokstad and B. L. Hutchings, Lederle Laboratories, Inc.

and 500 to 1000 cells were classified. The submaxillary and posterior cervical lymph nodes, thymus, and spleen were dissected clean, weighed in the wet state, and fixed in buffered 4 per cent aqueous formaldehyde for histologic study. Heart, lungs, liver, pancreas, adrenal glands, kidneys, right femur, and the thoracic portion of the spinal column were fixed similarly. The femur and spinal column were decalcified in 5 per cent formic acid. All these tissues were embedded in paraffin, sectioned, and stained with hemalum-azure-cosinate and with iron hematoxylin-picrofuchsin.

The sections of femur and spinal column were studied to determine the proportion of active marrow by a method described in detail in a previous report.<sup>1</sup> In brief, this consists of preparing 5" x 7" photomicrographs of the marrow portion of the midshaft, distal metaphysis, distal epiphysis of the femur and of the body of a thoracic vertebra under standard photographic conditions at a magnification of 250 diameters. These photomicrographs are analyzed to determine the areas occupied by active marrow, fat, vasa, and bone trabeculae. The proportion of active marrow is determined in each photomicrograph by the formula

$$\text{Proportion of active marrow} = \frac{\text{Area occupied by active marrow}}{\text{Available marrow space}}$$

The average of the four areas was determined and this was multiplied by the percent of each type of cell in the marrow smear to give an index of the total quantity of each type of cell. Some such quantitation is essential in making accurate comparisons of hemopoiesis, particularly where marked hypoplasia or hyperplasia is accompanied by marked changes in the marrow differential.

#### RESULTS

In the tables the results of the various studies are presented as mean values with standard deviations  $\left( S. D. = \sqrt{\frac{\sum (x)^2}{n-1}} \right)$ . Each phase of the riboflavin deficiency study will be dealt with separately and will be correlated with the corresponding phase of the previous folic acid deficiency study.

#### *Hemopoiesis in Granulocytopenia*

The hemograms, myelograms, and organ weights in 12 rats with marked riboflavin deficiency granulocytopenia (less than 150 neutrophils/cu. mm.) are shown in table 1. Graphic comparison of these rats with 13 rats showing similar blood picture as a result of folic acid deficiency is made in figure 1. In both deficiencies the findings were essentially the same.

The marrow was hypocellular, active marrow being largely replaced by dilated blood channels and edema fluid. Granulocytes of all types were markedly decreased in every rat. The average indexes of erythroid cells, blast cells, and lymphocytes were decreased. Slight to marked increases of plasma cells and reticulo-endothelial cells were observed.

Atrophy of the lymphoid apparatus and absence of splenic hemopoiesis accompanied the changes in the blood and bone marrow.

TABLE 1.—*Hemograms and Myelograms in Experimental Roflostin Deficiency Granulocytopenia before and after Treatment with Folic Acid*  
(Means and Standard Deviations)

	22 Normal adult rats	12 Rats untreated	8 Rats treated 4 days	6 Rats treated 8 days	7 Rats treated 12 days
Peripheral blood					
Hemoglobin Gm./100 cc. ....		13.5 ± 1.1	12.0 ± 2.0	10.0 ± 1.7	12.9 ± 2.5
Hematocrit. ....		42.7 ± 3.7	37.1 ± 4.7	36.9 ± 6.5	41.4 ± 8.9
Erythrocytes/cu. mm. in millions	45.6 ± 3.9	6.89 ± .63	6.15 ± 1.08	5.37 ± .96	7.36 ± 1.83
Leukocytes/cu. mm. ....	14,570 ± 5,110	3,080 ± 2,070	8,240 ± 2,594	9,006 ± 7,180	8,200 ± 3,530
Neutrophils/cu. mm. ....	3,180 ± 1,970	65 ± 43	2,180 ± 800	2,730 ± 1,775	2,274 ± 1,627
Eosinophils/cu. mm. ....	240 ± 171	0 ± 0	150 ± 340	x had 70, x had 100, others 0	x had 75, others 0
Lymphocytes/cu. mm. ....	10,840 ± 4,332	3,005 ± 2,160	5,490 ± 2,190	6,248 ± 5,888	5,951 ± 2,389
Nucleated erythrocytes per cu. mm. ....	x had 40, others 0	240 ± 666	260 ± 416	301 ± 465	156 ± 209
Reticuloocytes % .....		2.1 ± 2.1	2.9 ± 1.7	3.8 ± 2.0	1.4 ± 1.3
Proportion of active marrow .....	.827 ± .091	.436 ± .203	.582 ± .147	.819 ± .120	.772 ± .127
Index of total quantity in marrow*					
Neutrophilic segmented forms & metamyelocytes (Index) .....					
Neutrophilic myelocytes & premyelocytes (Index) ..	28.5 ± 6.0	2.3 ± 2.2	16.4 ± 9.9	25.2 ± 18.7	24.6 ± 6.9
Eosinophilic granulocytes of all types (Index) ..	7.6 ± 2.2	1.4 ± 1.1	6.2 ± 6.4	8.4 ± 5.0	7.1 ± 3.1
Total myeloid cells (Index) ....	6.9 ± 4.6	.3 ± .2	1.0 ± .7	.9 ± .7	.7 ± .1
Erythroid cells of all types (Index) ....	43.0 ± 10.5	4.1 ± 2.9	23.6 ± 13.7	34.5 ± 20.7	32.5 ± 8.2
Myeloid/erythroid ratio .....	25.5 ± 7.3	22.5 ± 15.5	19.1 ± 11.4	28.3 ± 11.0	32.3 ± 12.1
Blast cells of all types (Index) .....	1.93 ± .93	.41 ± .60	1.24 ± .62	.92 ± .52	1.11 ± .28
Lymphocytes of all types (Index) .....	1.6 ± 0.5	.6 ± .3	1.2 ± .63	1.7 ± .64	.8 ± .4
Miscellaneous cells† (Index) .....	11.0 ± 3.5	10.7 ± 5.2	9.5 ± 4.9	12.9 ± 5.5	6.0 ± 1.4
Body weight, Gm. ....	1.7 ± 1.0	5.6 ± 2.8	4.4 ± 2.0	4.9 ± 2.6	6.6 ± 3.5
Organ weights, mg.		52 ± 11.9	54 ± 11.4	53 ± 17.9	61 ± 12.7
Thymus .....		24 ± 13	27 ± 9.6	20 ± 9.5	26 ± 25.1
Spleen .....		323 ± 121.6	267 ± 79.0	393 ± 155.6	419 ± 158.7
Lymph nodes, submaxillary and posterior cervical ..		75 ± 35.8	110 ± 61.2	97 ± 25.8	114 ± 36.5

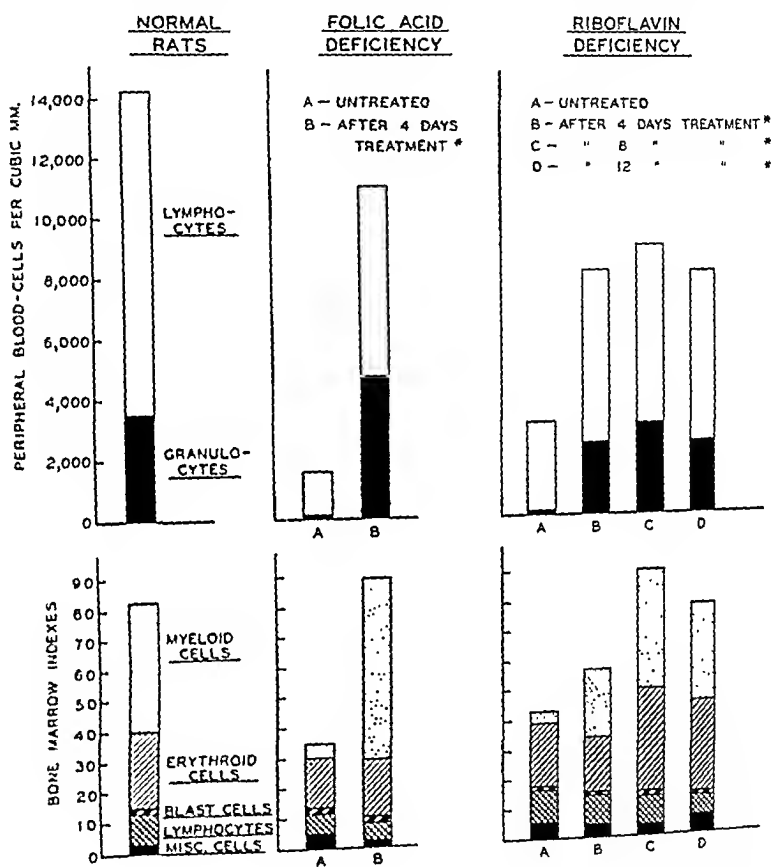
\* See the text for the method of determining the index.

† Reticulo-endothelial cells, plasma cells, mast cells, megakaryocytes, and unidentified cells.

Bacterial infections both localized and widespread were common and presumably may be attributed to the granulocytopenia and lowered resistance.

*Effect of Folic Acid on Hemopoiesis in Granulocytopenic Rats*

The detailed findings in riboflavin deficiency granulocytopenia after 4, 8, and 12 days' therapy with daily oral 25 microgram doses of crystalline folic acid are



\* TREATMENT CONSISTED OF DAILY ORAL DOSES OF 25 MICROGRAMS OF CRYSTALLINE FOLIC ACID.

FIG. 1. GRAPHIC COMPARISON OF BLOOD AND BONE MARROW IN FOLIC ACID AND RIBOFLAVIN DEFICIENCY GRANULOCYTOPENIA

shown in table 1. Graphic comparison of riboflavin-deficient rats and folic acid-deficient rats is presented in figure 1.

In both deficiencies, folic acid therapy of 4 days' duration restored the circulating neutrophils to normal levels, increased the cellularity of the bone marrow, and markedly increased the granulocytic indexes above the pretreatment levels. It is obvious, however, that the response to folic acid therapy in riboflavin-deficient

(Means and Standard Deviations)

	22 Normal adult rats	7 Rats with polymorphonuclears over 1,000/cu. mm.	4 Rats with polymorphonuclears between 500 & 1,000/cu. mm.	5 Rats with polymorphonuclears between 150 & 500/cu. mm.	13 Rats with polymorphonuclears less than 150/cu. mm.
Peripheral blood					
Hemoglobin Gm./100 cc.		15.8 ± 1.9	13.3 ± 1.1	12.5 ± 3.9	13.5 ± 1.1
Hematocrit	45.6 ± 3.9	49.7 ± 5.0	43.3 ± 6.5	40.0 ± 6.1	42.7 ± 3.7
Erythrocytes/cu. mm. in millions		7.99 ± 0.75	7.59 ± 1.45	6.08 ± 0.50	6.89 ± 0.63
Leukocytes/cu. mm.	14,570 ± 5,110	6,015 ± 2,230	5,254 ± 5,338	4,215 ± 1,817	3,080 ± 2,070
Neutrophils/cu. mm.	3,180 ± 1,970	2,170 ± 707	836 ± 90	296 ± 94	65 ± 43
Eosinophils/cu. mm.	240 ± 171	1 had 370, others 0	15 ± 19.2	1 had 15, others 0	0 ± 0
Lymphocytes/cu. mm.	10,840 ± 4,332	3,795 ± 2,339	4,402 ± 1,638	3,920 ± 1,800	3,005 ± 2,160
Nucleated erythrocytes per cu. mm.	1 had 40, others 0	0 ± 0	121 ± 446	515 ± 1,064	240 ± 666
Reticuloocytes %		0.9 ± 0.8	2.3 ± 3.3	1.3 ± 0.8	2.1 ± 2.1
Proportion of active marrow	0.827 ± 0.21	0.671 ± 0.174	0.759 ± 0.092	0.615 ± 0.133	0.436 ± 0.203
Index of total quantity in marrow*					
Neutrophilic segmented forms & metamyelocytes (Index)					
Neutrophilic myelocytes & premyelocytes (Index)	28.5 ± 6.0	21.9 ± 10.1	7.0 ± 13.6	5.8 ± 4.6	2.3 ± 2.2
Eosinophilic granulocytes of all types (Index)	7.6 ± 2.2	4.4 ± 2.7	3.9 ± 0.6	3.4 ± 1.9	1.4 ± 1.1
Total myeloid cells (Index)	6.9 ± 4.6	0.4 ± 0.4	0.9 ± 0.5	0.9 ± 0.7	0.3 ± 0.2
Erythroid cells of all types (Index)	43.0 ± 10.5	26.8 ± 12.6	18.4 ± 7.9	10.1 ± 5.0	4.1 ± 2.9
Myeloid/erythroid ratio	25.5 ± 7.3	23.3 ± 8.8	30.8 ± 4.3	35.0 ± 16.2	22.5 ± 15.5
Blast cells of all types (Index)	1.93 ± 0.93	1.38 ± 1.02	0.61 ± 0.27	0.37 ± 0.83	0.41 ± 0.60
Lymphocytes of all types (Index)	1.6 ± 0.5	1.0 ± 0.1	0.50 ± 0.14	1.00 ± 0.60	0.60 ± 0.30
Miscellaneous cellst (Index)	11.0 ± 3.5	11.7 ± 5.0	21.2 ± 12.1	10.3 ± 3.2	10.7 ± 5.2
Body weight, Gm.	1.7 ± 1.0	4.4 ± 1.5	5.0 ± 1.0	5.1 ± 1.5	5.6 ± 2.8
Organ weights, mg.		89 ± 46.9	53 ± 17.8	53 ± 15.3	52 ± 11.9
Thymus		28 ± 26.1	19 ± 11.1	17 ± 13.5	24 ± 13
Spleen		281 ± 220.2	241 ± 134.7	249 ± 94.4	323 ± 121.6
Lymph nodes, submaxillary and posterior cervical		85 ± 61.9	92 ± 34.3	56 ± 25.1	75 ± 35.8

\* See the text for the method of determining the index.

† Reticulo-endothelial cells, plasma cells, mast cells, megakaryocytes, and unidentified cells.

rats is less rapid and less complete than the response in folic acid-deficient rats.

In riboflavin-deficient rats the extension of the treatment period to 8 days produced further response of the granulocytopoietic tissues but not to the extent of the marked hyperplasia seen in some of the treated folic acid-deficient rats. After 12 days of folic acid treatment there was some suggestion that granulocytopoiesis might be decreasing to a subnormal level.

Hemopoiesis in the spleen reappeared in both deficiencies after folic acid therapy but was more marked in the group of treated folic acid-deficient rats than in the group of treated riboflavin-deficient rats.

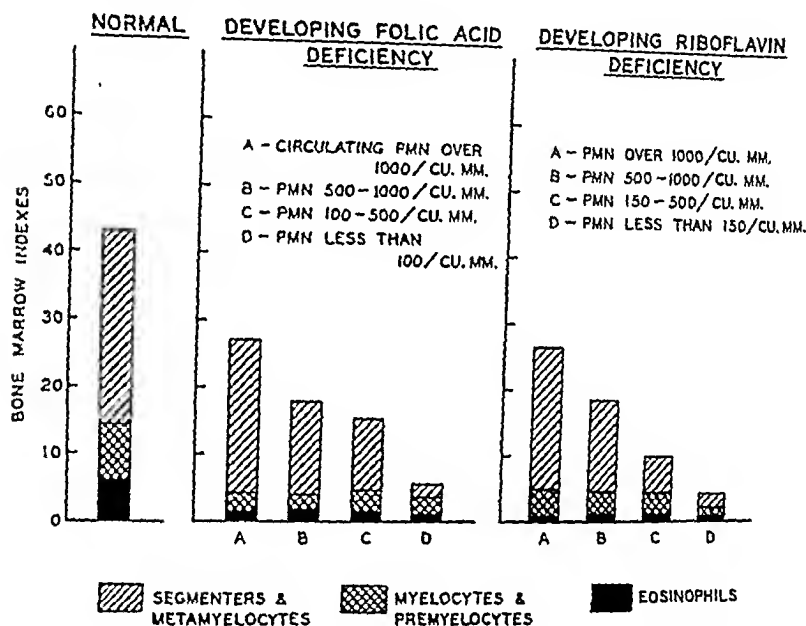


FIG. 2. GRAPHIC COMPARISON OF MYELOPOIESIS IN FOLIC ACID AND RIBOFLAVIN DEFICIENCY

Lymphoid structures showed some regeneration in the treated group of riboflavin-deficient rats. No comparison can be made since lymph nodes and thymus were examined in only a few rats in the study on folic acid deficiency.

#### *Hemopoiesis in Developing Granulocytopenia*

Detailed findings are shown in table 2 and comparative graphs of developing granulocytopenia are shown in figure 2. In both deficiencies there was progressive depletion of cells of the granulocytopoietic series in the marrow. The closely parallel nature of these changes in the two deficiencies is evident in the chart. The myeloid depletion appeared while the number of circulating granulocytes was still within the normal range. With increasing granulocytopenia in both deficiencies the total myeloid index fell from a normal average of 43.0 to an average of less than 5 in rats whose circulating neutrophils numbered less than 150 per cubic millimeter.

It is evident that the depletion of the bone marrow was largely restricted to the granulocytic series, although some erythroid depletion occurred in the advanced stages of the granulocytopenia.

TABLE 3.—*Hemograms and Myelograms in Experimental Riboflavin Deficiency Granulocytopenia and Anemia before and after Treatment with Riboflavin*  
(Means and Standard Deviations)

	11 Rats untreated	4 Rats (blood picture before treatment)	4 Rats after 10 days' riboflavin therapy
Peripheral blood			
Hemoglobin Gm./100 cc. ....	6.0 ± 2.1	7.3 ± 2.1	9.3 ± 4.0
Hematocrit .....	21.0 ± 6.8	26.7 ± 3.7	32.8 ± 13.4
Erythrocytes/cu. mm. in millions. ....	3.15 ± 1.02	3.61 ± 2.06	3.66 ± 1.07
Leukocytes/cu. mm. ....	2,620 ± 1,959	7,500 ± 2,902	4,600 ± 2,361
Neutrophils/cu. mm. ....	110 ± 104	735 ± 1,020	805 ± 705
Eosinophils/cu. mm. ....	0 ± 0	30 ± 58	25 ± 11
		1 had 115, others 0	
Lymphocytes/cu. mm. ....	2,310 ± 1,568	6,735 ± 1,939	3,745 ± 1,045
Nucleated erythrocytes per cu. mm. ....	810 ± 734	1,360 ± 1,161	1,195 ± 2,019
Reticulocytes % .....	6.5 ± 5.9	7.2 ± 4.1	42.7 ± 37.9
Proportion of active marrow. ....	.625 ± .202		.871 ± .069
Index of total quantity of marrow*			
Neutrophilic segmented forms & metamyelocytes (Index) .....	2.8 ± 2.7		12.4 ± 13.3
Neutrophilic myelocytes & premyelocytes (Index) .....	3.3 ± 2.9		6.6 ± 2.6
Eosinophilic granulocytes of all types (Index) .....	0.7 ± 0.6		2.6 ± 2.2
Total myeloid cells (Index) .....	6.8 ± 4.8		21.7 ± 17.5
Erythroid cells of all types (Index) ..	40.2 ± 17.4		54.7 ± 13.9
Myeloid/erythroid ratio. ....	0.20 ± 0.13		0.49 ± 0.62
Blast cells of all types (Index) .....	1.6 ± 1.0		1.0 ± 0.62
Lymphocytes of all types (Index) ..	8.9 ± 3.2		4.4 ± 1.0
Miscellaneous cells† (Index) .....	4.8 ± 2.7		5.3 ± 1.2
Body weight, Gm. ....	46 ± 11.3		81 ± 6.9
Organ weights, mg.			
Thymus. ....	17 ± 9.3		78 ± 44.8
Spleen .....	181 ± 94.7		803 ± 483.5
Lymph nodes, submaxillary and posterior cervical .....	83 ± 51.7		133 ± 54.4

\* See the text for the method of determining the index.

† Reticulo-endothelial cells, plasma cells, mast cells, megakaryocytes, and unidentified cells.

The average weights of lymphoid structures were low even in those animals with more than 1000 neutrophils per cubic millimeter. This might be expected in view of the fact that the rats were uniformly lymphocytopenic. Histologically the lymph nodes were often devoid of germinal centers or these structures were very small, showed few mitotic figures, and were without collars of small lymphocytes. Thymuses were atrophic or showed acute pyknotic involution in all but 2 rats.

These were in the group having over 1000 neutrophils/cu. mm. Changes in lymphoid structures appear to be unrelated to the level of circulating neutrophils.

### *Hemopoiesis in Combined Granulocytopenia and Anemia*

Hemograms and myelograms of riboflavin-deficient rats showing granulocytopenia (less than 150 neutrophils/cu. mm.) and anemia (hematocrit less than 30 vol. per cent) are shown in table 3. The bone marrow in such rats showed depletion of the granulocytic series but the erythrocytic series was actually increased and in

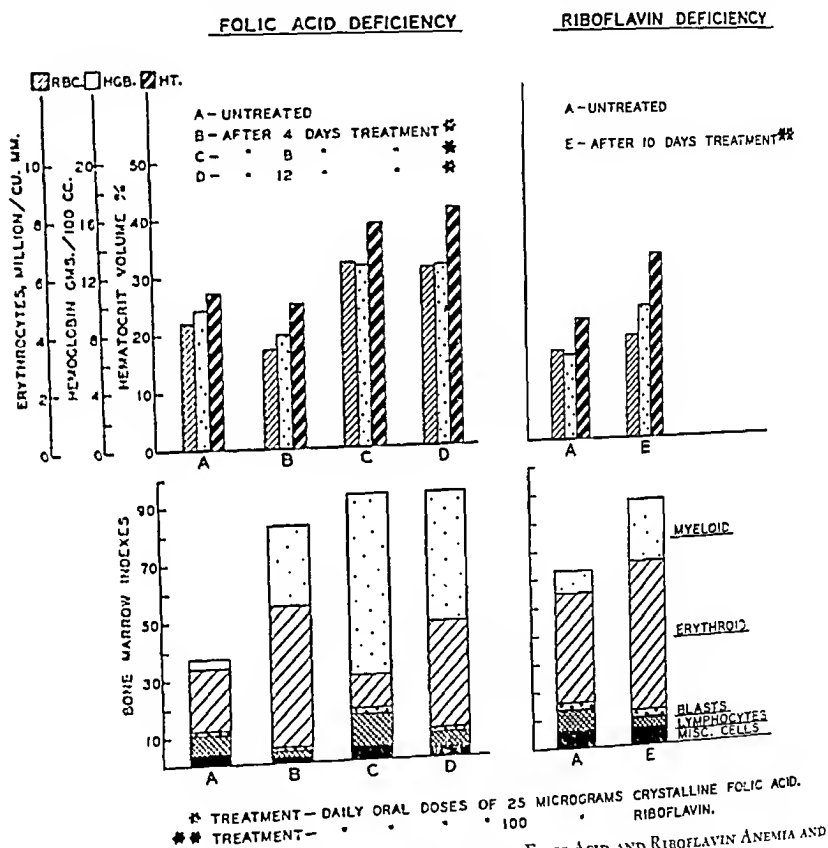


FIG. 3. GRAPHIC COMPARISON OF BLOOD AND BONE MARROW IN FOLIC ACID AND RIBOFLAVIN ANEMIA AND GRANULOCYTOPENIA

several rats the index of erythroid cells was over 60 as compared to an average normal index of 25.5.

This erythroid hyperplasia in riboflavin deficiency anemia contrasts sharply with the erythroid hypoplasia of folic acid deficiency anemia. Hemopoiesis in the two deficiencies is compared graphically in figure 3.

### *Effect of Riboflavin on Hemopoiesis in Riboflavin Deficiency Anemia*

The hemograms and myelograms of 4 rats treated for 10 days with daily oral doses of 100 micrograms of riboflavin are shown in table 3 and figure 3. We wish to

emphasize the fact that these rats were only slightly granulocytopenic and therefore the myelogram cannot be compared to that of untreated rats which were both anemic and granulocytopenic. The response as measured in the blood appears to be quite definite with some elevation of hemoglobin, hematocrit, and erythrocyte and reticulocyte counts. The degree of lymphocytopenia, however, increased under riboflavin therapy while the granulocyte level remained essentially the same. In the marrow there was marked erythroid hyperplasia. Active splenic hemopoiesis, large active lymphoid follicles in spleen and lymph nodes, and histologically normal noninvolved thymus were noted in all 4 rats. Unfortunately the very low incidence of anemia in our experiments prevented our securing data on sufficient numbers of rats. Additional anemic rats are being studied as they become available and are being treated with riboflavin, with folic acid, and with both.

#### COMMENT

Riboflavin deficiency under the conditions of our experiments produced granulocytopenia and myeloid hypoplasia which was indistinguishable from the granulocytopenia and myeloid hypoplasia of folic acid deficiency. The hematopoietic response of these riboflavin-deficient rats to folic acid therapy was prompt and quite marked. This situation is somewhat paradoxical. The diet is adequate if riboflavin is added, and yet when riboflavin is withheld signs and symptoms of folic acid deficiency appear which respond to folic acid therapy. It seems likely that riboflavin deficiency produces this effect by reducing the food intake with resultant protein deficiency. The intimate relationship of protein deficiency and folic acid deficiency has been demonstrated<sup>5</sup> but the underlying physiology remains to be explained.

With respect to the granulocytopenia which accompanies riboflavin deficiency, it seems reasonable to state that the feeding of a highly purified diet deficient in riboflavin has produced a folic acid deficiency. With respect to the anemia which is seen less frequently than, but usually associated with, granulocytopenia, the explanation is much more obscure. In primary folic acid deficiency we found that the bone marrow showed depletion of the erythroid series in granulocytopenic rats whether anemia was present or not. One would expect, therefore, that in folic acid deficiency caused by riboflavin deficiency the same situation would obtain. This was not found to be the case. In rats with granulocytopenia but without anemia there was depletion of erythroid cells in the marrow. In rats with granulocytopenia and anemia there was an absolute increase of erythroid cells in the marrow. Furthermore, preliminary experiments have shown that folic acid therapy is without effect upon the anemia but that riboflavin therapy is effective. Although further work is necessary before one can be certain it appears that the anemia is a direct result of riboflavin deficiency.

In other species of animals riboflavin deficiency has produced anemia. Spector and co-workers<sup>9</sup> found anemia in dogs maintained on riboflavin-deficient diets. Wintrobe and co-workers<sup>10</sup> have reported anemia in riboflavin-deficient swine, while Waisman<sup>11</sup> found leukopenia and anemia in riboflavin-deficient monkeys. The monkeys continued to eat well even in advanced deficiency so that protein inanition does not appear to have been a factor.

## SUMMARY

Leukopenia, granulocytopenia, and, occasionally, anemia develop in rats fed purified diet deficient in riboflavin. Folic acid (*L. casei* factor) corrects the leukopenia and granulocytopenia. Riboflavin will prevent all the dyscrasias but will correct only the anemia.

The bone marrow in granulocytopenic rats is hypoplastic and is almost completely depleted of cells of the granulocytic series. Cells of the erythroid series are decreased in number. The myelogram of rats made folic acid deficient by the inclusion of sulfasuxidine in a purified diet resembles this picture and in both cases the response of the marrow and the blood to folic acid therapy is similar.

The bone marrow in riboflavin-deficient rats having both granulocytopenia and anemia is depleted of granulocytic cells but shows an erythroid hyperplasia. This myelogram differs from that seen in sulfasuxidine-induced folic acid deficiency anemia and granulocytopenia in which there is erythroid hypoplasia. The two anemias differ further in that the folic acid deficiency anemia responds to folic acid therapy whereas the riboflavin deficiency anemia responds to riboflavin therapy but not to folic acid therapy.

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# THE HEMATOLOGIC RESPONSE IN DOGS TO THE ADMINISTRATION OF ANTI SPLEEN SERUM

By JOHN B. MIALE, M.D.

SINCE the early days of medicine, when each organ had its real or fancied attributes, the idea of restoring function to a deranged part by the administration of tissue from a normal organ has seemed attractive. However little the effort then availed, the principle has in recent years given organotherapy many important agents such as insulin and liver extract.

With the development of the science of immunology it seemed only natural that, instead of using organs *per se*, attempts be made to produce an antiserum to each. Certainly if such a preparation had any effect, either stimulant or depressant, there would be opened up a new field of almost unlimited possibilities.

The problem was defined by Metchnikoff,<sup>34</sup> who called such antisera "cytotoxic" in the belief that their effect on the cells of the body was a toxic one. He injected splenic tissue into animals in an attempt to make an antispleen serum, but soon abandoned this work, primarily because of the difficulties in determining what, if any, titer of antiserum he was working with. Funck,<sup>19</sup> Flexner,<sup>18</sup> and Bunting<sup>10</sup> used other tissues, such as lymph nodes and bone marrow, as antigens with indifferent results. More recently, in 1936, Chew, Stephens, and Lawrence<sup>13</sup> reported that an antileukocytic serum produced in some guinea pigs an almost complete reduction in the number of neutrophil leukocytes in the peripheral blood. Earlier work by Ledingham and Bedson,<sup>24</sup> Bedson,<sup>1</sup> Lindstrom,<sup>26</sup> Okita,<sup>35</sup> and Matsuno<sup>29</sup> had given essentially similar results. Although this effect was striking, a satisfactory technic for titrating the antisera was still not available.

The present study was stimulated by the recently reported Russian work in which a new technic for the titration of "cytotoxic" serum was used. Marchuk,<sup>27, 28</sup> Bogomolets and his colleagues have named this serum "anti-reticular cytotoxic serum." The claims made for its therapeutic application are many.<sup>2-9, 11, 12, 14, 17, 20, 22, 23, 25, 30-33, 36, 37, .</sup> Although they are yet to be confirmed, and the mode of action elucidated, the standardization of a method of preparation of antisera and for their titration offers renewed hope that fundamental problems of hematology and allied fields can be attacked with this new tool.

## MATERIALS AND METHODS

### I. Experimental Animals

Dogs and rabbits were used for this study. The animals were kept in individual cages, the temperature and humidity in the animal quarters controlled at all times. When first obtained, all animals were kept in "isolation" quarters for a period of two weeks and observed for infection. Healthy animals were then moved to the

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main quarters, placed on a standard diet, and again observed for several weeks. During this time those animals were chosen for the experiment which ate the diet well, maintained body weight, and exhibited good vitality. During the period of observation, these animals were free from any known infection, and remained so during the experiment.

*Dogs.*—Male hounds 1-2 years of age and weighing 5-10 Kg. were selected. The standard diet was Purina Dog Chow. Water was given ad lib.

TABLE 1. *The Effect of Dosage and Route of Administration of Antigen on Titer of Antisera Obtained—Rabbit Anti-Dog-Spleen Serum*

Rabbit	Weight (Kg.)	Antigen	Route	Dosage—Gm. of Tissue Equivalents	Titer of Antiserum Obtained 14 Days after Last Injection
44-2	2.80	Spleen	Intraperitoneal	Day 1—0.1 Day 6—0.2 Day 11—0.3 Day 16—0.4 Day 21—0.6	1:160
44-10	3.13	Spleen	Intravenous	Day 1—0.01 Day 6—0.02 Day 11—0.04 Day 16—0.04 Day 21—0.08	1:640
44-11	3.24	Spleen	Intravenous plus Intraperitoneal	Day 1—0.01 i.v. 0.12 i.p. Day 6—0.02 i.v. 0.20 i.p. Day 11—0.02 i.v. 0.30 i.p. Day 16—0.02 i.v. 0.40 i.p. Day 21—0.04 i.v. 0.50 i.p.	1:320
44-12	3.36	Spleen	Intravenous	Day 1—0.01 Day 6—0.02 Day 11—0.04 Day 16—0.04 Day 21—0.08	1:400

*Rabbits.*—White litter-mate chinchilla rabbits about 1 year of age and weighing 2-3 Kg. were used. They were fed Rockland Rabbit Diet with added vegetables.

## II. Preparation and Titration of Antisera

The method will be outlined in detail only for the preparation of antispleen serum as it is the same for heart, bone marrow, and other antigens.

Under ether anesthesia the dogs were bled out and spleens removed. The splenic capsule was trimmed away, 5.0 grams of spleen sliced thin with a razor and washed gently in five changes of physiological saline to remove as much blood as possible.

The tissue was then thoroughly ground in a mortar with sterile, pure sea sand, resuspended in 25.0 cc. of physiological saline, and centrifuged for 4 minutes at 1000 rpm. The top fatty layer was pipetted off and discarded, and the supernate used as antigen. This material was used both for injecting rabbits and for the complement fixation test. Antigen so obtained was designated "undiluted antigen," and contained 0.2 gram of spleen/cc.

# APPENDIX—Titration of Antisera

## A. Determination of Titer of Antigen

	1	2	3	4	5	6	7	8
Dilution of antigen	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.
Titrated complement	1.0 cc.	1.0 cc.	1.0 cc.	1.0 cc.	1.0 cc.	1.0 cc.	1.0 cc.	1.0 cc.
Saline	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.

### 37° C. Water Bath—45 Minutes

Sensitized sheep cells	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
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### 37° C. Water Bath—30 Minutes

Note 1. Antigen is prepared as for immunization.

Note 2. Complement previously titrated to contain 2 full units in 1.0 cc.

Note 3. Sensitized sheep cells: 2% suspension with previously titrated hemolysin.

Note 4. Titer of antigen to be used is the smallest quantity which gives complete fixation of complement.

## B. Determination of Titer of Antiserum

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Antiserum	1:20	1:50	1:100	1:200	1:300	1:400	1:500	1:600	1:700	1:800	1:900	1:1000	1:2000	1:3000
	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Titrated antigen	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Titrated complement	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

### 37° C. Water Bath—45 Minutes

Sensitized sheep cells 2%	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
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### 37° C. Water Bath—30 Minutes

Note 1. Serum is inactivated at 56° C. for 30 minutes before the test is carried out.

Note 2. Titer of antiserum is highest dilution causing complete fixation of complement.

Note 3. Standard controls are set up.

Note 4. The hemolytic titer of the antiserum is also determined and sera with titers of 1:16 or more are not used.

Rabbits were injected by various routes, but the highest titers of antiserum were obtained when the antigen was given intravenously (table 1). Every fifth day the dose was increased (table 1), and each time fresh spleen was used in preparing the antigen. Fourteen days after the last injection 5.0 cc. of blood was obtained by cardiac puncture and the antispleen titer of the serum determined by a modification of Marchuk's method (Appendix).

Rabbits showing a high titer were bled out and the serum separated with careful consideration for a sterile technic. It was found that allowing the blood to clot and storing it in the refrigerator overnight gave the highest yield of serum. This

was pipetted off and stored in sterile 1 cc. glass ampules at 4 degrees centigrade. Sterility of the serum was checked several times, and always before using.

### III. Administration of Antiserum

The serum was diluted 1:10 with sterile physiological saline 48 hours before administration and stored in the refrigerator while sterility was being checked by the inoculation of veal infusion broth and blood agar culture media. In this study the serum was given intravenously. The dosage was calculated on the basis of 0.1 cc. of 1:10 diluted serum/10 Kg. of body weight for the first dose, the second dose being three times the first.

TABLE 2.—Data on a Dog (44-23) Receiving Antispleen Serum AS-10 Intravenously

Date	Time	Weight (Kg.)	HGB. (G. %)	RBC (millions)/cu. mm.	WBC/cu. mm.	% Lymphocytes	% Monocytes	Lymphocytes/cu. mm.	Monocytes/cu. mm.	Total Mononuclears/cu. mm.	Sedimentation Rate mm./hr.	Volume Packed RBC %	Reticulocyte %	Remarks
11/29/44	1:00 P.M.	6.45	18.0	8.5	10,500	22	3	2300	310	2610	1 mm.	52	1%	(Platelet counts, blood chemistry values, etc., were normal and are not shown)
11/30/44	2:30 P.M.	6.45	18.5	9.1	9,100	25	5	2280	455	2735	1 mm.	53	<1%	
12/1/44	3:30 P.M.	6.37	17.5	8.8	9,850	15	4	1775	395	2170	—	53	—	
12/2/44	1:43 P.M.	6.54	18.0	8.8	11,100	22	6	2440	666	3106	—	—	—	
12/3/44	2:00 P.M.	6.30	18.0	9.5	11,250	21	5	2360	565	2925	1 mm.	51.5	<1%	
12/4/44	2:30 P.M.	6.35	17.5	9.3	10,000	26	6	2600	600	3200	—	50	1%	0.1 cc. 1:10 AS-10 i.v. at 5 P.M.
12/5/44	2:30 P.M.	6.46	17.4	8.46	12,900	35	3	4500	378	4878	—	50	—	
12/6/44	2:00 P.M.	—	—	—	12,150	35	7	4250	850	5110	1 mm.	51	<1%	0.3 cc. 1:10 AS-10 i.v. at 5 P.M.
12/7/44	2:15 P.M.	6.40	17.5	8.45	10,850	30	5	3260	545	3805	—	49	—	
12/8/44	2:15 P.M.	6.35	15.2	7.54	10,750	37	4	3460	430	3890	—	—	—	
12/9/44	2:30 P.M.	6.28	16.0	8.39	10,250	49	6	5025	615	5640	1 mm.	48	<1%	
12/11/44	2:15 P.M.	6.15	—	—	10,250	34	6	3480	615	4095	1 mm.	—	—	
12/13/44	2:00 P.M.	6.39	16.8	9.44	10,250	35	8	3600	820	4420	—	48	<1%	
12/15/44	2:30 P.M.	6.38	17.8	8.81	10,150	28	7	2840	710	3550	—	—	—	
12/18/44	2:30 P.M.	6.16	17.6	8.89	10,050	31	3	3120	300	3420	—	47	1%	
1/3/45	3:00 P.M.	6.39	17.5	8.52	8,250	24	4	1980	330	2310	—	47	—	
2/7/45	2:00 P.M.	6.4	17.0	8.95	10,000	23	4	2300	400	2700	1 mm.	47	<1%	

### IV. Experimental Procedures

All food was taken out of the dog cages in the evening and studies were carried out at about 2 o'clock the following afternoon before the dogs were fed again. Dogs were easily trained to lie quietly on the table. Blood samples were obtained by jugular puncture, care being taken to avoid venostasis. Smears on cover slips and supravital preparations were immediately made with fresh blood, and 10.0 cc. of blood was oxalated with dry potassium and ammonium oxalate as recommended by Heller and Paul.<sup>21</sup> The fixed blood smears were stained routinely with Wright's blood stain, and occasionally with special stains where indicated. The supravital technic employed combined Janus green and neutral red as the stain. Differential

TABLE 3.—*Summary of Results*

Dog	Experiment	Serum and Titer	Dosage (cc. undiluted serum)	Lymphocytes/cu. mm.					Monocytes/cu. mm.					Total Mononuclears/cu. mm.				
				Average (control period)	Average first 6 days	% Change Average	Peak Level	% Change Peak	Average (control period)	Average first 6 days	% Change Average	Peak Level	% Change Peak	Average (control period)	Average first 6 days	% Change Average	Peak Level	% Change Peak
44-1	Antispleen serum i.v.	AS-10 1:500	0.015—Day 6 0.045—Day 8 0.135—Day 11 0.405—Day 13 1.21—Day 15	1590	3291	+107	4780	+200	411	414	0	1020	+148	2000	3673	+84	4694	+134
44-23	Antispleen serum i.v.	AS-10 1:500	0.01—Day 6 0.03—Day 8	2290	4100	+79	5025	+119	500	565	+11	850	+70	2790	4664	+67	5640	+98
44-26	Antispleen serum i.v.	AS-10 1:500	0.012—Day 6 0.036—Day 8	2113	3389	+61	3825	+72	915	2046	+123	2360	+147	3028	5435	+80	6210	+105
44-18	Antispleen serum i.v.	AS-10 1:500	0.012—Day 6 0.036—Day 8	2965	5115	+71	6000	+102	540	1231	+127	1680	+211	3500	6346	+82	7660	+119
44-31	Antispleen serum i.v.	AS-10 1:320	0.015—Day 6 0.045—Day 8	2023	2368	+17	2620	+30	543	1064	+96	1311	+141	2656	3432	+29	4045	+58
45-2	Antispleen serum i.v.	AS-12 1:400	0.012—Day 6 0.036—Day 8	2940	3235	+10	3920	+33	277	708	+155	1660	+500	3217	3943	+23	4920	+53
45-4	Antispleen serum i.v.	AS-12 1:400	0.01—Day 6 0.03—Day 8	3263	2880	-13	3380	+4	266	1096	+310	1435	+440	3529	3976	+13	4590	+30
44-29	Control normal rabbit serum i.v.	R-15 0	0.0135—Day 6 0.0405—Day 8	3874	3626	-7	4370	+13	915	930	+2	1090	+2	4789	4556	-5	4920	+3
44-35	Control normal rabbit serum i.v.	R-17 0	0.015—Day 6 0.045—Day 8	3372	3022	-11	3780	+12	621	625	0	830	+34	3993	3646	-9	4340	+9
44-30	Control normal rabbit serum i.v.	R-21 0	0.016—Day 6 0.048—Day 8	2876	2900	+1	3285	+14	428	400	-7	825	+93	3304	3300	0	3655	+11
44-36	Control anti-heart serum i.v.	AH-1 1:500	0.01—Day 6 0.03—Day 8	4575	4370	-5	4990	+7	464	545	+17	910	+96	5039	4515	-12	5534	+10
44-37	Control anti-heart serum i.v.	AH-1 1:500	0.01—Day 6 0.03—Day 8	3722	3444	-8	3950	+4	744	761	+2	980	+32	4466	4205	-6	5020	+15
45-18	Antimarrow serum i.v.	AM-1 1:400	0.006—Day 6 0.018—Day 8	2780	2540	-9	3000	+8	612	1131	+85	1760	+187	3392	3671	+8	4180	+23
44-22	Large dose antispleen serum i.v.	AS-10 1:500	2.25—Day 6	4780	3400	-41	1900	-153	625	490	-28	136	-360	5490	3890	-42	2036	-123

counts on 200 leukocytes were done on each preparation. Blood cell counts were carried out in duplicate and sometimes triplicate, and for each dog the same pipets

and chambers were used at all times. The type of data obtained for each dog is shown in table 2.

After a preliminary period of six days for base line determinations, 7 dogs received antispleen serum, 3 dogs received normal rabbit serum having no antispleen titer, and 2 dogs received antiheart serum. One dog received antimarrow serum, and 1 dog received a single large dose of antispleen serum (table 3, col. 2).

#### RESULTS

The results are summarized in table 3.

The only marked effect noted was a relative and absolute increase in the mononuclear cells of the peripheral blood. A marked lymphocytosis was seen, and also an increase in monocytes. From fixed smears it was sometimes impossible to classify some of the mononuclear cells as either lymphocytes or monocytes, and parallel supravitral studies showed the cells in question to have the large mitochondria of lymphocytes and the neutral red rosette typical of monocytes. For the purpose of analyzing the results, therefore, these cells were considered together as "mononuclear cells."

As will be seen from table 3, the 4 dogs receiving rabbit anti-dog-spleen serum AS-10 with an antispleen titer of 1:500 showed increases in absolute numbers of circulating mononuclears of 134 per cent, 98 per cent, 105 per cent, and 119 per cent. Dog 44-31 received the same serum which, after it had been stored at 4° C. for 5 months, showed diminution of titer to 1:320. The mononuclear response in this case was only +58 per cent. Dogs 45-2 and 45-4 received a second lot of antispleen serum, AS-12, made in the same way and having a titer of 1:400. These showed increases of 53 per cent and 30 per cent.

The control animals which received normal rabbit serum having no antispleen titer, and those receiving an antiheart serum (AH-1) having an antiheart titer of 1:500 and an antispleen titer of 0, showed no corresponding mononuclear response. The maximum peak reached was 15 per cent above the base line.

The rise in circulating mononuclears in very prompt, occurring within 24-48 hours of the first dose of serum, and under the conditions of the experiment there is a return to the base line figure in about 20 days. The major portion of the rise is due to cells which are typically lymphocytic, and it was noted that there were relatively more of the large variety during the height of the response. Later an increase in typical monocytes was observed, while the period in between was characterized by cells smaller than monocytes and having the ambiguous features already noted. In 1 dog (44-1) these cells of doubtful classification reached at one time a level of 12 per cent. An inconstant feature was the occasional appearance during the height of the mononuclear response of large blast cells which lacked sufficient criteria for classification.

There were no other significant changes. The hemarocrit values remained constant or became slightly lower, and there was never any evidence of hemoconcentration. Occasionally moderate decreases in the red blood cell count were seen, accompanied by lower hemoglobin values, but the icterus index did not increase

and there was no change in the reticulocyte count. In some dogs the titer of complement in the blood was followed through the period of mononuclear response without any increase being noted. In 2 dogs daily determinations of the heterophil antibody titer failed to show any rise. Blood chemistry values (NPN, albumin, globulin, fibrinogen) remained constant. Sedimentation rates were normal in all experimental dogs and did not change during the experiments. The white blood count showed occasional slight rises during the mononuclear response, while with equal frequency it became somewhat lower. There was no significant increase in blood platelets, although 1 dog in the antispleen group showed at one time a platelet count of 4.5 millions.

The effect of a large dose of antispleen serum was studied in dog 44-22. This dog was given 22.5 cc. of 1:10 diluted serum, equivalent to 5 cc./10 Kg. This dose was somewhat toxic, as evidenced by vomiting produced two hours after it was given intravenously. The dog refused to eat his diet that evening, but seemed to be well the next morning. In this case the lymphocyte percentage in the peripheral blood dropped from a base line average of 27 per cent to 7 per cent two days later, and at no time showed the rise in mononuclears seen in the dogs receiving small doses. Calculated on the basis of absolute numbers of mononuclear cells, the change in this case was -123 per cent. At the same time a marked eosinophilia was noted.

#### DISCUSSION

The future significance of these findings is necessarily in the realm of speculation. Two principles, however, the specificity of antisera and the opposite effects of small and large doses, are supported by the data obtained.

What is the mode of action of "cytotoxic" serum? That such small doses should have any action whatever seems remarkable, and yet many substances necessary for cellular integrity and function are measurable as traces rather than in grams. The vitamins, for instance, acting as activators, catalysts, or even precursors, exert effects out of proportion to the extremely small amounts in which they are active. This is even more striking in the case of the "trace" elements and the hormones. Considerable evidence has accumulated to show that large amounts are unnecessary, and may even be toxic.

The first conclusion to be drawn from the data presented, supported by previous reports on antisera of other types, is that, if the serum is active at all, the action is fairly specific. Only one cell type, or one group of related cells, is affected, and the results depend on the antigen used. Thus Chew, Stephens, and Lawrence<sup>13</sup> made an antileukocytic serum by injecting cells from experimentally produced peritoneal exudates into rabbits. This antigen consisted of 75-90 per cent neutrophils, 1-4 per cent lymphocytes, the remainder being large mononuclear cells. Proportionately large doses of this serum (the antileukocytic titer in vitro was not determined) when given intravenously to guinea pigs produced in a few hours an almost complete disappearance of neutrophils from the peripheral blood, the other cells being relatively unaffected. Yamamoto<sup>14</sup> using serum from a goose immunized against rabbit neutrophils also found the effect to be entirely on the neutrophils. Experi-

ence with antiplatelet serum by many observers has shown that it is specific for platelets. In the present study it is notable that the lymphocytes, monocytes, and mononuclear cells were affected, corresponding to the main cellular population of the spleen. Serum made against heart muscle had, by contrast, no effect on these cells.

The second conclusion is that the dosage of antiserum given is of special importance, and that small doses have a different effect from large doses. When rabbit anti-dog-spleen serum is given in very small doses (total of 0.04 cc./10 Kg.) the mononuclear cells in the peripheral blood show increases of 100 per cent or more. If a large dose is given (5 cc./10 Kg.) a reduction of 123 per cent results. It should be emphasized that in the previously quoted work on antileukocytic serum,<sup>13</sup> relatively large doses of serum were used to produce depression of the neutrophils (1.3-2.3 cc. Kg.). The work of Bogomolets is based on the assumption that small doses are "stimulating" while large doses are "blocking" in effect. The difference in response in dogs to the administration of small and large quantities of antispleen serum is partial confirmation of this.

If this assumption is correct, it should be possible to either stimulate or depress a cellular system at will by administering small or large doses, respectively, of a specific antiserum. The therapeutic applications would be many. For example, an increase in mononuclear cells in the peripheral blood is often a good prognostic sign in acute infectious diseases. The same is generally true in chronic infections. In tuberculosis, for example, not only is the lymphocyte/monocyte ratio important, but also the polynuclear/mononuclear ratio. Ratios and indices, such as those of Sabin and Medlar, are attempts to express this numerically. The large mononuclear cells of the blood and fixed tissues are responsible for the formation of the inner cellular portion of the tubercle, the small lymphocytes mark its outer border, while resolution or fibrosis is probably a function of the same mononuclear cells. Therefore, any agent which stimulates this group of cells to greater activity might be an adjuvant to general or specific therapy.

In the field of acute infections, any agent which stimulates antibody production in the infected host is beneficial. Interesting in this connection are the recent reports by Dougherty, White, and Chase,<sup>15</sup> Ehrlich,<sup>16</sup> and White<sup>18</sup> indicating that antibodies are probably made or stored in the lymphocytes. Again it might be hoped that stimulation of these cells would result in increased antibody formation.

Many other possibilities suggest themselves. The lack of specificity of morphological methods has, it is well known, led to considerable confusion in the interpretation of cellular origins, relations, and reactions. After many years of careful study by outstanding investigators, the "reticulo-endothelial system," for example, is still the subject of spirited controversy. The same may be said of the various theories on the origins of blood and tissue cells. It is hoped that specific stimulation or inhibition by means of antisera may be the biological "tool" which is needed to clarify these fields.

In making this preliminary investigation of antispleen serum, many questions have been raised. It is to be hoped that further work will provide the answers.

## SUMMARY

1. Intravenous injection into dogs of rabbit anti-dog-spleen serum in doses of 0.04 cc./10 Kg. results in a significant increase in the mononuclear cells of the peripheral blood.
2. The rise in circulating mononuclears occurs promptly, with 24-48 hours, and is sustained, generally for 20 days.
3. A large dose of the same serum, 5.4 cc./10 Kg., exerted an opposite effect, producing a significant decrease in circulating mononuclears.
4. The possible significance of these findings is discussed.

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# CONTRIBUTION TO THE PATHOGENESIS OF HEMOPHILIA

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IN STUDYING the mechanisms which alter normal hemostasis in hemophilia consideration must be given to the two principal factors, since both appear to be involved, although in different proportions.

The alterations in the hematic factor, represented by a delayed coagulation, have puzzled a great number of competent investigators. Alterations in the vascular factor have been less seriously considered, although in our opinion they play a definite role.

In this paper we shall refer to the various studies on hemophilic coagulation, which is characterized by a lengthening of the coagulation time, apparently caused by a deficit in the thromboplastic factor. To explain this deficit there are three acceptable theories:

## (1) *Slow liberation of thromboplastin by an excessive stability of the platelets*

This theory was supported by Sahli (1910),<sup>1</sup> Fonio (1914-36),<sup>2,3</sup> Minot and Lee (1916),<sup>4</sup> Howell and Cekada (1926),<sup>5</sup> Fiessinger and Letard (1940),<sup>7</sup> and was refuted by Feissley (1923),<sup>8</sup> Patek and Stetson (1936),<sup>9</sup> Howell (1939),<sup>10</sup> and Quick (1942).<sup>11</sup> (See also Tocantins.<sup>6</sup>)

## (2) *An actual deficiency of thromboplastin*

Schmidt in 1893 (quoted by Quick<sup>11</sup>) suggested that the coagulation defect in hemophilic blood is determined by a deficiency in thromboplastin, since zymoplastic substances shorten the coagulation time of hemophilic blood. This theory was subsequently supported by Sahli (1905),<sup>12</sup> Weil (1906),<sup>13</sup> Morawitz and Lessen (1908),<sup>14</sup> Kottmann and Linsky (1910),<sup>15</sup> Schloessmann (1912),<sup>16</sup> and others.

In 1936 new investigations were begun in the United States and Holland (Bendien and Van Creveld<sup>17</sup>) which supported this latter theory. The researches carried out in the Thorndike Memorial Laboratory began with the study of Patek and Stetson,<sup>9</sup> who were supported later by Taylor,<sup>18-22</sup> Pohle, Lözner, and Kark.<sup>23,24</sup> Finally Lewis, Tagnon, Davidson, Minot, and Taylor<sup>25</sup> summarized this work and concluded that in normal whole blood, or platelet-free plasma, there exists a substance (globulin fraction) which shortens the coagulation time of hemophilic blood. The same fraction extracted from hemophilic plasma contains little coagulant power, from which they inferred that hemophilic blood was deficient in some factor or factors present in normal blood. They termed this fraction "anti-hemophilic globulin."

Howell in 1939<sup>10</sup> called this fraction "thromboplastin" instead of globulin but he agreed that hemophilic blood contains a diminished quantity of thromboplastin.

## (3) *Increase of anticoagulant substances which inhibit normal thromboplastin activity*

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The presence of anticoagulant substances has been mentioned by several investigators although their precise nature and their exact mode of action have remained largely obscure. Weil in 1906<sup>13</sup> discovered an increase of antithrombin in hereditary hemophilic blood, which did not exist in sporadic cases. This observation could not be confirmed by Sahli.<sup>1</sup> According to Laclette,<sup>26</sup> "Hydeck of Bratislava in 1923 considered that the most important factor in hemophilia was an excess of antithrombin, the production of which might be under the control of the sexual glands." Feissly in 1924<sup>8</sup> suggested the presence in hemophilic blood of a substance which could prevent the normal transformation of prothrombin into thrombin. On the other hand, in 1931 Evans and Howell<sup>27</sup> discarded the theory of an excess of anticoagulants, either antithrombin or antiprothrombin. Chaher (1935)<sup>28</sup>

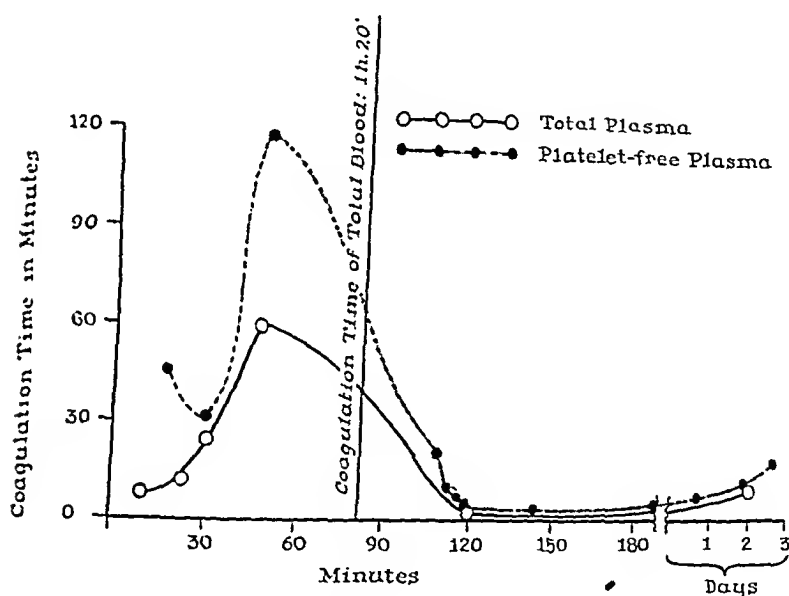


FIG. 1. PERIOD BETWEEN COLLECTION AND RECALCIFICATION OF BLOOD

also disbelieved in the existence of anticoagulant substances. Fonio (1936)<sup>3</sup> in his review concluded that it had not been possible to prove the presence of these anticoagulant substances. Lawrence and Johnson (1942)<sup>29</sup> observed that the blood of a hemophilic patient, added to that of another hemophilic, lengthened the coagulation time of the latter. They maintained that the former's blood contained an anticoagulant substance which was neither heparin nor antithrombin.

In 1942 Tocantins<sup>30</sup> suggested the possibility of the presence of antithromboplastic substances in hemophilia. In 1943<sup>31</sup> he verified the presence of antithromboplastic substances in both normal and hemophilic blood. He maintained that these antithromboplastic substances acted during the first stages of coagulation, and were later neutralized by the coagulant factors, which are formed in the last stages. It is the presence of these factors that may explain the difficulties found in identifying the

antithromboplastic substances. This antithromboplastic substance may be exhaustible, having a different degree of specificity in the various species; it becomes inactive when heated to 65° C. for 5 minutes; its activity decreases slowly if kept in paraffined or lusteroid tubes. Tocantins found that hemophilic plasma has an anticoagulant activity from five to eight times greater than that of normal plasma. The author termed this substance "anticephalin." In a more recent paper Tocantins<sup>32</sup> gave details of his investigations by which he demonstrated that the increased stability (tendency to remain fluid) of hemophilic blood is due to an uncompensated excess of anticephalin activity.

In 1942 Quick<sup>31</sup> maintained that "the existence of an agent which can neutralize thromboplastin has never been established, nor have any substances been found or prepared which can antagonize by direct action the activity of this clotting factor. This negative evidence does not preclude the possibility that such an agent may ultimately be found." In 1944 Quick<sup>33</sup> seemed to support the theory that the antithromboplastin present in normal blood is abnormally increased in hemophilic blood.

From our own observations, we consider that anticoagulant substances are present in hemophilic blood. These probably act on the coagulant globulin fraction diminishing its activity.

#### OBSERVATIONS

In 1941, in collaboration with M. R. Castex,<sup>34</sup> we determined the clotting time of recalcified plasma of hemophilic blood, and noted variations in some cases, according to the time at which the test was determined. If the determination was made immediately after collection, the coagulation time of hemophilic recalcified plasma was longer than that of normal plasma; but if the test was made some time after the collection, a period that varied according to the coagulation time of the patient's whole blood, it was definitely shorter. Similar results were obtained by Quick in 1941.<sup>11</sup>

At first we attributed this shortening to a slow disintegration of the hemophilic platelets, which freed the thromboplastin with equal slowness. This hypothesis was discarded after making the same test with platelet-free recalcified plasma, when the same diminution in coagulation time was noted and which could, therefore, not be attributed to the platelets. That indicated the possibility that some anticoagulant substance might be present in hemophilic plasma, which would become inactivated, leaving free the coagulant factors, which would then act as in normal blood.

Subsequently,<sup>35</sup> following the observations of Patek and Stetson,<sup>9</sup> we fixed the optimal doses of total blood, plasma, or serum required to reduce the clotting time of each patient. It was found that the amounts of material required varied with the clotting time of the hemophilic blood, so that for a longer coagulation time, the injection of a larger quantity of normal blood, plasma, or serum was required. One of our patients required very large amounts of blood (more than 600 cc.) in contrast with others who required only 140 cc.

Following observations *in vitro* (in collaboration with Castex and Simonetti<sup>36</sup>)

we verified the influence of the proportion of sodium citrate used in the solutions added to the blood. When instead of using a 3.8 per cent solution (1 to 10 of blood) we used a 7.5 per cent solution of this salt, an anticoagulant effect was noted. In all further experiments we therefore used the 3.8 per cent solution.

With the same collaborators we studied the action of the hemophilic blood added to the blood of other hemophilic patients, and observed a paradoxical fact. We found that occasionally (in vitro) the blood of some of the hemophilic patients with a greatly prolonged clotting time (1 hr. 20 mins.) when added to other hemophilic blood with a much shorter clotting time (40 mins.) possessed a coagulant action nearly as effective as normal blood. Acting on this observation, we transfused plasma from the patient with the longest clotting time to one of the other patients (table 1). The results of this experiment made us doubt whether a deficiency in a coagulant substance (globulin fraction) really existed; for if it did exist, it would be illogical to obtain a coagulant action with hemophilic blood which contained a smaller quantity of coagulant substance.

TABLE 1.—*Transfusion of 100 cc. of Plasma from Hemophilic 1 to Hemophilic 9*

	Hemophilic H <sub>9</sub> Hemophilic H <sub>1</sub>	Coagulation time: 30 minutes Coagulation time: 80 minutes	
		Time elapsed after the transfusion of plasma from hemophilic H <sub>1</sub>	Coagulation time of hemophilic H <sub>9</sub>
Before the transfusion			30 minutes
1½ hour later			6 minutes
24 hours later			13 minutes
48 hours later			25 minutes

If we admit that hemophilic blood has a deficit of globulin fraction, we consider that this deficit might be caused not by an original deficiency, but by the action of anticoagulant substances that would render it inactive. With the idea of studying this hypothesis we tried to diminish the coagulant action in normal persons in the help of anticoagulant substances. By using heparin by injection or dicoumarol orally (Pavlovsky and Simonetti<sup>27</sup>) we showed that the globulin fraction had less coagulant activity when coagulation was most delayed. From this experiment we deduced that if the globulin fraction of the hemophilic blood had less coagulant activity, it might be due to the presence of anticoagulant substances that would neutralize its action.

In further studies<sup>28</sup> we determined the coagulant activity of the globulin fraction in different patients and found that it varied, but not in accordance with the length of clotting time. In some of our patients with a greatly prolonged clotting time, the globulin fraction possessed a coagulant activity almost equal to that of normal blood. This indicated the possibility that if an anticoagulant fraction existed, it had a different stability or a different activity in each patient; thus in some the proportion might be greater than in others, hence their longer or shorter clotting time. This substance might become inactive in varying periods of

time after the blood has been extracted, this being the cause of the discordant variations in the coagulant activity of the globulin fraction in the different hemophilic patients.

With this thought in mind, we tried to obtain inactivation of the anticoagulant substance by precipitating the globulin fraction of the hemophilic plasma, in two ways. One sample was obtained by precipitating the globulin immediately after collection of the blood, the other, by leaving the blood 24 hours in a refrigerator before precipitating the protein fraction. In the second test we verified that in some cases the globulin fraction had increased its coagulant power. We thought that this might be due to the inactivation of the anticoagulant fraction.

In accordance with the preceding experiment, we gave our patients autotransfusions of blood, verifying that if we extracted the blood and injected it again immediately afterwards no modification was obtained in the coagulation time; but if the blood was injected after having been kept in a refrigerator for 48 hours, a coagulant effect was obtained that varied according to the patient. We proved that the effect was more obvious if we used whole blood instead of plasma.

From the foregoing evidence, we deduced that it might be possible to solve the problem of the coagulation time in hemophilia by inactivation of anticoagulant substances. It has been established that these anticoagulant substances are neither heparin nor dicoumarin.

We tried also (in vitro) to shorten the clotting time of hemophilic blood by the addition of protamine, without attaining a satisfactory result. It is generally acknowledged that protamine reduces the anticoagulant activity of heparin (Chargaff and Olson,<sup>39</sup> Jaques and Waters,<sup>40</sup> and Jorpes<sup>41</sup>). Despite these negative results, we believe that investigations should be continued to discover a medium that would inactivate these hypothetical anticoagulant substances.

This will be the aim of our further investigations.

#### SUMMARY

The causes of the delayed coagulation of hemophilic blood seem to become clearer as time advances. On one side we have the investigations of the school of Minot, indicating a deficit in the globulin fraction; and on the other side are the works of those who maintain that there exists an excess of anticoagulant substances. We support the latter theory, although in our opinion the two theories do not contradict each other, since it might be possible that this anticoagulant substance would act on the globulin fraction diminishing its coagulant power. This substance could be identified with the anticephalin fraction of Tocantins.

With the idea that this substance might be less stable than the coagulant fractions (fibrinogen, prothrombin, and thromboplastin) we have tried to render it inactive by keeping the blood in a refrigerator for a certain length of time. Inactivity was obtained in some of our experiments. We know that the stability of this substance varies from one patient to the other, but we have not been able to fix the cause of these variations.

In conclusion, we consider that other means of neutralizing the action of this

anticoagulant substance should be investigated. This inactivity once obtained, we should have advanced far in solving the intricate problem of hemophilia.

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# RENAL FUNCTION DURING CHRONIC ANEMIA IN MAN

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**P**HYSIOLOGICAL adjustments to slowly developing anemia are so successful that anemic patients characteristically present themselves with only minor complaints of fatigability, weakness, or dizziness, despite severe reductions of the hematocrit to 15 per cent or less. These adjustments are largely cardiovascular.<sup>1</sup> Oxygen transport is maintained by a marked augmentation of blood flow through most parts of the body as a result of peripheral vasodilatation and increased cardiac output.<sup>2</sup> Oxygen uptake by the tissues is more efficient. Initially the compensation for the reduction of the red cell mass is adequate and effective, but, ultimately, limitations become apparent. Congestive heart failure may develop, possibly as a result of myocardial anoxia,<sup>3</sup> and renal dysfunction may appear, with azotemia, proteinuria, and isosthenuria.<sup>4, 5</sup>

The renal circulation has been found to play an important role in the cardiovascular adjustments during fever, fear, orthostasis, and shock.<sup>6, 7</sup> In acute blood loss and shock<sup>6</sup> active renal arteriolar constriction appears to divert blood from the kidney to regions such as the heart and brain where it is more urgently required. The present study indicates that, in chronic anemia, a similar active renal vascular response occurs and probably interferes with renal function.

## SUBJECTS AND METHODS

The subjects of this study were 15 patients with severe chronic anemia. Eight were proved cases of pernicious anemia in relapse who responded to therapy with liver extract. The other 7 suffered from anemia due to other causes: 2 had paroxysmal nocturnal hemoglobinuria, 2 had bleeding duodenal ulcers, and 1, bleeding hemorrhoids. One patient had lymphatic leukemia, proved by hematological study and subsequent postmortem examination, and 1 was anemic as a result of iron deficiency.

The sexes were about equally divided, although females dominated the group with pernicious anemia, 6 to 2, while males outnumbered females among those with secondary anemia by 6 to 1. The age range was wide, ranging from 24 years to 72 years, but a third of the cases were more than 60 years of age. This preponderance of older subjects appeared to have little influence upon the results.

Without exception these patients had been moderately active until the time of admission to the hospital. Most complained of dyspnea on exertion and a moderate degree of dizziness and faintness on standing. The anemia and the symptoms referable to it were of at least two weeks' duration, and in most instances had been present for several months. It seems safe to assume that all patients were physiologically adjusted to the severe grades of anemia they presented.

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An effort was made to exclude subjects in whom factors other than anemia might have interfered with renal function, but it is possible that other processes were active in 3. In 1 patient with pernicious anemia, L. M., a persistent arterial hypertension was found. It should be noted that there has been no evidence of progression of the hypertensive disease over a three year period of observation and that cardiac, ocular, or cerebral involvement has not occurred. While it is possible that in the 2 subjects with paroxysmal nocturnal hemoglobinuria the prolonged excretion of hemoglobin might have produced renal parenchymal damage, the renal functional changes were in general agreement with those of the other cases (table 1).

All subjects were studied in the fasting basal state, following the ingestion of 1500 to 2000 cc. of water to provide adequate urine flow. Kidney function was measured with the technics devised by H. W. Smith and his co-workers, summarized by Goldring and Chasis<sup>8</sup> in their monograph, *Hypertension and Hypertensive Disease*. Glomerular filtration rate was determined as the mannitol\* or inulin clearance; and effective renal plasma flow, as the diodrast clearance. Effective renal blood flow was calculated from the diodrast clearance and the hematocrit. Tubular function was studied by saturation technics,<sup>8</sup> the maximal tubular excretory capacity with diodrast (diodrast Tm) and maximal tubular reabsorptive capacity with glucose (glucose Tm). Analyses were performed upon plasma filtrates, prepared according to the method of Fujita and Iwatake,<sup>9</sup> and diluted aliquots of urine. Diodrast, mannitol, and inulin were determined by methods outlined by Goldring and Chasis<sup>8</sup>; glucose, by that of Nelson.<sup>10</sup>

Although all subjects were studied thoroughly from the hematological point of view, and, in many instances, followed for a period of years in the Hematology Clinic, only the hematocrit values will be presented in this paper. These values were obtained by centrifuging oxalated blood in Wintrobe tubes in duplicate, at 3000 revolutions per minute for 60 minutes.

#### RESULTS

Table 1 summarizes the results of the various tests of renal function in the 15 subjects. All the values collated in table 1 are averages of two or more determinations. As a rule, three or more figures were obtained but in several instances values were discarded for technical reasons. All figures are expressed in terms of the surface area of ideal man ( $1.73 \text{ M.}^2$ ) in order to minimize differences attributable to variation in size. With 4 exceptions, all subjects were studied prior to treatment. Two and three transfusions had been given to L. T. and M. B., respectively, during the week prior to the renal function test. Two patients with pernicious anemia had received liver extract: K. Mc. for two weeks and M. Ch. for nine days.

Seven patients (table 2) were studied prior to and, on various occasions, after

\*Mannitol, ampuled in 25 per cent sterile solution, was supplied for use in this study through the courtesy of the Medical Research Division of Sharp and Dohme, Inc., Philadelphia, Pennsylvania. Inulin (10 per cent in sterile solution), manufactured by William R. Warner and Company, New York, and diodrast (35 per cent in sterile solution), manufactured by the Wirthrop Chemical Company, New York, were used in this study.

effective treatment was started. Hematologic improvement was noted in all, in 3 (E. M., L. M., and H. C.) the hematocrit returning to normal by the time of

TABLE 1.—*Renal Function in Chronic Anemia*

Each datum in columns 5 to 12 represents the average of two or more clearance periods and is corrected to 1.73 sq. M. Glomerular filtration rate denotes values of mannitol and inulin clearances; effective renal plasma flow, values of diodrast clearances.

Subject	Age	Sex	Body Surface	Glomerular Filtration Rate (G.F.R.)		Effective Renal Plasma Flow (R.P.F.)		Filtration Fraction (G.F.R./R.P.F.)		Effective Renal Blood Flow (R.B.F.)		Diodrast Tm (Tmd)	R.B.F./Tmd.	Glucose Tm (Tmg)		G.F.R./Tmg	Hct.		Arterial Blood Pressure	Diagnosis
				M <sup>2</sup>	ml./min.	ml./min.	per cent	ml./min.	mg./min.	mg./min.	per cent			mm. Hg.						
E. M.	57	F	1.40	118	609	19.6	699		699		34	24.2					12.8	114/66	Pernicious anemia	
K. Mc.	63	F	1.63	83	604	13.7	820		820		31	14.2					26.4	112/54	Pernicious anemia	
M. C.	57	F	1.48	58	351	16.8	440		440		31	14.2					20.3	94/64	Pernicious anemia	
R. deV.	65	M	1.67	121	575	20.8	695		695		46	15.1	222	0.544			17.3	140/65	Pernicious anemia; diabetes mellitus	
A. K.	72	M	1.89	79	457	17.7	580		580		21	27.6	289	0.273			21.3	150/50	Pernicious anemia; arteriosclerotic cardiovascular disease	
M. Ch.	37	F	1.66	137	523	26.3	646		646				461	0.297			19.0	114/60	Pernicious anemia	
L. M	67	F	1.86	68	381	18.4	528		528								27.7	180/98	Pernicious anemia; essential hypertension	
H. C.	64	F	1.62	119	353	21.4	705		705		34	20.7	433	0.364			21.6	130/70	Pernicious anemia; arteriosclerotic cardiovascular disease	
L. T.	24	M	1.77	26	203	12.8	277		277								26.7	124/58	Paroxysmal nocturnal hemoglobinuria	
L. G.	27	M	1.78	76	752	10.1	887		887		26	34.1					15.2	106/64	Paroxysmal nocturnal hemoglobinuria	
H. Cu.	47	M	1.71	94	640	14.6	816		816		31	26.3					21.6	110/70	Chronic blood loss—hemorrhoids	
M. B.	31	M	1.96	108	501	22.1	672		672		68	9.9					25.5	96/48	Chronic blood loss—duodenal ulcer	
H. S.	38	M	1.72	80	528	15.1	648		648		34	19.1	349	0.229			18.6	114/66	Iron deficiency	
B. K.	56	F	1.50	144	716	19.9	877		877				415	0.347			18.4	124/66	Lymphatic leukemia	
J. S.	53	M	1.86	125							42		387	0.323			28.2	110/80	Chronic blood loss—duodenal ulcer	
Average values—female—normal <sup>13</sup> .....				117	594	20.2	982	42.6	982	23.0	303	0.395								
Average values—female—anemia .....				104	534	19.4	673	33.0	673	19.7	436	0.356								
Average values—male—normal <sup>13</sup> .....				131	697	19.0	1209	51.8	1209	25.3	375	0.371								
Average values—male—anemia .....				89	522	16.2	654	38.3	654	22.0	312	0.342								

the last observation. On the whole, follow-ups such as these proved difficult to obtain since the subjects refused to return to the hospital for study when they

felt completely well. The follow-up studies summarized in table 2 covered various periods of time ranging from two weeks to about three years of observation.

### *Effective renal blood flow*

The most striking functional alteration during chronic anemia regardless of etiology was the reduction of effective renal blood flow. This alteration in effective whole blood flow was associated with a much smaller change in the effective

TABLE 2.—*Renal Function During and Following Recovery from Chronic Anemia*

Each datum in columns 3 to 8 is an average of two or more clearance periods (except one, marked with an asterisk) and is corrected to 1.73 sq. M. The glomerular filtration rate denotes values of mannitol and inulin clearances, and effective renal plasma flow, values of diodrast clearances.

Subject	Date	Glomerular Filtration Rate (G.F.R.)	Effective Renal Plasma Flow (R.P.F.)	Filtration Fraction G.F.R./ R.P.F.	Effective Renal Blood Flow (R.B.F.)	Diodrast Tm (Tmd)	R.B.F./ Tmd	Hct.	Blood Pressure
		ml./min.	ml./min.	per cent	ml./min.	mg./min.		per cent	mm. Hg.
E. M.	11/17/42	118.0	609	19.6	699			12.8	114/66
	12/18/42	109.0	477	23.0	645	32.0	20.1	25.9	138/72
	2/22/43	76.1	422	18.0	715	37.0	19.3	41.3	112/68
	6/21/43	90.5	575	15.7	1048	44.7	23.5	45.1	120/70
L. M.	11/19/42	68.0	381	18.4	528			27.7	180/98
	12/ 2/42	92.3	388	23.6	578	18.4	31.4	32.9	120/77
	12/21/42	90.0	420	21.4	680	16.5*	41.2	38.3	138/74
M. C.	6/15/43	58.0	351	16.8	440	31.0	14.2	20.3	94/64
	8/ 3/43	74.6	326	22.9	423	31.0	13.6	29.7	110/70
H. C.	1/ 2/46	119.0	553	21.4	705	34.0	20.7	21.6	130/70
	5/15/46	117.0	642	18.2	927			30.6	124/70
H. Cu.	12/22/42	94.0	640	14.6	816	31.0	26.3	21.6	110/70
	1/ 4/43	52.4	397	13.3	724	28.2	25.2	44.2	94/50
L. G.	2/ 1/43	76.0	752	10.1	887	26.0	34.1	15.2	106/64
	10/ 3/45	119.9	594	20.2	856			30.6	104/70
H. S.	2/15/43	80.0	528	15.1	648	34.0	19.1	18.6	114/66
	3/10/43	95.5	579	16.6	685	36.0	19.0	15.5	120/60
	6/18/43	93.4	635	14.7	936	45.0	20.7	32.2	124/70

renal plasma flow, the latter always falling in the lower portion of the normal range. On the average, the effective volume of whole blood flowing through the kidney fell from a normal figure of 1209 ml./min. to 654 ml./min., or 46 per cent in males, and from 982 ml./min. to 673 ml./min., or 31.8 per cent in females. The diodrast clearance or effective renal plasma flow, on the other hand, decreased in males by 25.2 per cent from the normal figure of 697 ml./min. to 522 ml./min., and in females by 10.5 per cent from 597 ml./min. to 534 ml./min. The clearance method

may be used to measure renal plasma flow only if 90 to 100 per cent of the diodrast (or sodium p-aminohippurate) in the blood traversing the kidneys is removed. Since there is evidence that the extraction of diodrast or p-aminohippurate is not reduced during anemia,<sup>11</sup> these values are probably accurate and valid.

The collected group of females showed a considerably smaller average change in effective renal plasma flow than the males. This difference was also observed with regard to the values for whole blood flow, glomerular filtration rate, and maximal tubular capacities. Whether this difference in response represents a true sex difference cannot be said upon the basis of this small series. Since most of the women had pernicious anemia it is possible that the typical functional pattern is less striking in pernicious anemia. However, B. K., a female with a severe secondary anemia of several months' duration, also presented high figures. No correlation between the extent of renal hemodynamic change and duration of symptoms was found.

In view of the fact that cardiac output is almost always increased in anemia of this severity<sup>2</sup> it follows that the fraction of cardiac output passing through the kidneys fell markedly. This decrease was not only relative to blood flow elsewhere in the body, but absolute, since the fall in renal blood flow was considerably larger than the change in the head of pressure in the renal artery. Thus, the average mean arterial pressure (calculated according to the method of Böger and Wezler<sup>12</sup>) fell only 7.8 per cent, or from an average normal figure of 90 mm. Hg. to 83 mm. Hg. in both men and women, excluding L. M. Consequently, it is probable that active vasoconstriction within the renal vascular bed accounted for the reduction of blood flow.

Following treatment, the renal blood flow slowly improved as the hematocrit returned toward normal (table 2). This increase was clearly related to the hematocrit since the effective renal plasma flow (or diodrast clearance) showed relatively little change. Indeed, on two occasions (E. M. and H. C.) where determinations were made at short intervals, the effective renal plasma flow actually fell during recovery from anemia despite a rise in effective renal blood flow. In most cases the effective renal plasma flow tended to increase slightly as recovery progressed. It should be noted that the arterial pressure did not increase.

#### *Glomerular filtration rate and the filtration fraction*

In more than half the subjects the glomerular filtration rate was below the normal range; in only 2 was it higher than the normal mean value. On the average it fell from a normal figure of 131 ml./min. in males to 89 ml./min. and 117 ml./min. to 107 ml./min. in females. The filtration fraction or percentage of plasma filtered at the glomerulus fell into the lower portion of the normal range or below it in nearly every case.

As anemia responded to treatment the glomerular filtration rate tended to increase, although on several occasions it fell sharply during the early phase of recovery (table 2). The filtration fraction showed relatively little change. In most instances (best seen in E. M., H. C., and H. S.) it remained low. In 3 patients the filtration fraction returned toward the normal mean value but did not exceed it.

*Tubular function*

The maximal rate of tubular diodrast excretion (diodrast Tm) was determined in 10 subjects, 7 of whom had pernicious anemia. This value, normally 51.8 mg./min. in men and 42.6 mg./min. in women, was depressed significantly in 9 subjects; 22.5 per cent in females, and 39 per cent in males. This reduction could not be attributed to a failure to attain sufficiently high plasma concentrations of diodrast, for the load\* of diodrast presented to the tubules for excretion, in 8 of the 10, was greater than 2.0 mg. for each unit of Tm. In H. C. and M. B. the load/Tm ratio was 1.44 and 1.77 respectively, probably sufficient to assure saturation.

Maximal tubular glucose reabsorption (glucose Tm) was measured in 7 patients; 4 with pernicious anemia and 3 with anemia of varying etiology. Unlike diodrast Tm, glucose Tm fell within the normal range in every subject, except one (R. de V.). It is of interest that this patient suffered from diabetes mellitus in addition to per-

TABLE 3.—*Glucose Loading and Maximal Tubular Reabsorptive Capacity (Tm)*

Each datum is an average of two or more clearance periods and is presented without correction for body surface. See footnote, below for discussion.

Subject	Glucose Load	Glucose Tm (Tmg)	Load/Tmg
	mg./min.	mg./min.	
H. S.	463.0	346.6	1.34
M. Ch.	479.9	442.4	1.08
R. de V.	362.0	214.5	1.69
A. K.	432.0	314.8	1.37
B. K.	620.0	359.6	1.72
L. G.	628.0	362.6	1.73
J. S.	647.5	415.9	1.55
H. C.	675.0	404.0	1.68

nicious anemia. With one exception (M. Ch.—1.08) the glucose load/Tm ratio exceeded 1.2, indicating the probability of effective saturation loads.

\*Loading of the tubular transfer mechanisms must be in excess of their capacity if accurate measurements of Tm are to be made. The load of glucose reaching the tubules for reabsorption (mg./min.) may be calculated as the arterial plasma concentration of glucose (mg./ml.) multiplied by the filtration rate (ml./min.). The diodrast load (mg./min.) is the plasma concentration of diodrast (mg./ml.) times the effective renal plasma flow (ml./min.) less the quantity of diodrast filtered at the glomerulus (plasma concentration of diodrast [mg./ml.] multiplied by the filtration rate [ml./min.] and by a factor of 0.72 to correct for protein-binding). Since high plasma levels of diodrast must be used in the determination of diodrast Tm, the effective renal plasma flow is estimated from the filtration fraction, determined during a control study, and the filtration rate, observed during Tm determination.

All measurements of diodrast and glucose Tm are tabulated in tables 3 and 4. These figures are presented as they were obtained, without correction for body surface, together with the loads and load/Tm ratios. It can be seen that, with few exceptions, the ratios exceeded 2.0 for diodrast and 1.2 for glucose, the lower limits of adequate loading set down by Smith<sup>12</sup> In the one instance (M. Ch., table 3) where the glucose load/Tm ratio was low, the Tm value was quite high and probably correct. In H. C. (table 4), with ratios of 1.44 and 1.91 for diodrast, the Tm values measured at different times agreed closely despite the divergence of ratios. M. B. (table 4), with a diodrast load/Tm ratio of 1.77, had the highest diodrast Tm observed.

On 5 occasions (E. M., L. M., M. C., H. C., and H. S.) diodrast Tm was determined at different times after treatment was started. In 2 (E. M. and H. S.) there was a definite change toward the normal value. The others revealed no significant change. In 1 of these (H. C.) only twelve days had elapsed and there was evidence, in the renal hemodynamic pattern, of continuing renal functional abnormality

TABLE 4.—*Diodrast Loading and Maximal Tubular Excretory Capacity (Tm)*

Each datum (except those marked with an asterisk) is an average of two or more clearance periods and is presented without correction for body surface.

Subject	Diodrast Load mg./min.	Diodrast Tm (Tmd) mg./min.	Load/Tmd
L. M.	69.2	19.8	3.50
	63.9*	17.8*	2.80*
E. M.	79.7*	25.9	3.25
	55.6	29.9	1.86
	103.8	36.2	2.87
H. Cu.	44.1*	30.6	1.44
	53.2	27.9	1.91
L. G.	123.0*	26.9	4.57
H. S.	99.6	33.7	2.93
	104.4	35.7	2.93
	90.7	44.9	2.02
K. Mc.	169.0*	32.0	5.28
M. C.	65.6*	26.5	2.56
	59.6	26.6	2.24
M. B.	135.8*	76.8	1.77
R deV.	94.0*	44.6	2.11
A. K.	99.4*	23.4	4.25
J. S.	87.3*	45.3	1.93
H. C.	132.0	32.0	4.12

despite hematologic cure. In another (M. C.) the hematocrit increased from 20.3 per cent to 29.7 per cent without any change in diodrast Tm.

#### DISCUSSION

It has been found that a characteristic and reversible renal functional abnormality develops in chronic anemia. Both effective renal plasma flow and glomerular filtration rate are reduced significantly, the latter to a somewhat greater extent in each case so that the filtration fraction (percentage of plasma filtered at the glomerulus)

tends to fall slightly. The most striking change is the marked reduction of effective whole blood flow through the kidneys. Since systemic arterial pressure does not change significantly while cardiac output increases, it is evident that active vasoconstriction occurs within the renal vasculature. In addition to the renal hemodynamic adjustment during anemia, abnormalities of renal tubular activity appear. The maximal excretion of diodrast is diminished while maximal glucose reabsorption remains unchanged. This dissociation indicates a local dysfunction of tubular cells rather than the destruction or inactivation of nephrons.<sup>14</sup>

The normal value for glucose Tm indicates that all the glomeruli continue to function and to provide glucose for reabsorption by the tubules. Hence it is evident that vasoconstriction does not entail shunting of blood away from renal parenchyma or the cessation of flow in any significant portion of the renal vascular bed.\* Vasoconstriction may be more intense in some areas but this phenomenon is not manifest in the data presented above. To detect activity of this character, titration studies<sup>13</sup> would be required.

Since blood continues to perfuse all the glomeruli, the filtration fraction may be used as a means of evaluating the site of vasoconstriction in the kidney. Accepting the hypothesis<sup>15</sup> that filtration equilibrium is approximately reached across the glomerular membrane, a fall in both filtration fraction and renal blood flow implies increased afferent arteriolar resistance. The filtration fraction denotes the extent to which the plasma proteins are concentrated by filtration, and since the plasma oncotic pressure is equal to the hydrostatic pressure in the capillaries at equilibrium, the filtration fraction is a function of the equilibrium pressure. The slight reduction in filtration fraction therefore indicates a slight fall in equilibrium pressure. In some subjects the equilibrium pressure apparently remained at a normal level. This means that the difference of pressure between the renal artery and the point of filtration equilibrium changed very little despite a marked reduction of blood flow. Hence, the resistance between these points, to which the afferent arterioles make the largest contribution, increased in a manner roughly proportional to the reduction of blood flow. In addition, the equilibrium pressure is the head of pressure at the beginning of the postglomerular vascular bed. In view of the smaller reduction in filtration fraction than in renal blood flow, it may be concluded that the forces opposing perfusion increased, possibly as a result of efferent arteriolar vasoconstriction. Consequently, it is probable that both afferent and efferent arteriolar vasoconstriction occur in chronic anemia, and since the filtration fraction tends to fall it may be surmised that afferent vasoconstriction is the more prominent.

The reduction of blood flow through the kidneys is presumably a homeostatic

\*The reduction of effective renal blood flow relative to the glucose Tm implies a diffuse and widespread renal ischemia during chronic anemia. However, no consistent change in the perfusion of functioning excretory tissue was observed. The effective renal blood flow/diodrast Tm ratio was elevated in 3 patients, reduced in 3, and normal in 3 (table 1). During recovery, the ratio increased in 2 individuals (E. M. and I. M., table 2) and remained unchanged in 3 others. Hence, no conclusion can be reached regarding the relationship between diodrast Tm and blood flow, although it is possible that they may have varied independently.

device by which blood is diverted to tissues with less resistance to oxygen lack. The amount of blood thus spared is considerable. Normally 1200 ml. of blood pass through the renal vascular bed each minute in men. The average reduction to 600 ml. observed in this series of patients (males) indicated a diversion of 600 ml. of blood per minute. Actually the saving was much greater, for the kidney might have been expected to participate in the general vasodilation. This phenomenon is not peculiar to chronic anemia for it occurs in traumatic shock,<sup>7</sup> orthostasis,<sup>16</sup> and Addison's disease.<sup>17</sup> A reduction of the effective circulating blood volume is a common denominator in these otherwise diverse conditions, but it is impossible at present to attribute renal vasoconstriction to this factor. Since the kidney is subject to direct stimulation only by the arterial pressure, blood composition, and, possibly, the nervous system, there is no obvious *point d'appui* at which effective hypovolemia alone might excite renal vasomotion. Blood volume alteration, as such, would not influence renal blood flow except through some intermediate secondary effect. Certainly, a secondary change in the arterial pressure cannot be adduced as the immediate cause, since the mean pressure may rise (as in orthostasis) or fall (as in shock). The possible role of humoral or neural activity resulting from a reduction of the circulating blood volume remains to be elucidated.

The clearance and  $T_m$  values showed a tendency to return to normal during treatment and after the return of the blood picture to normal. The release of renal vasoconstriction and the correction of the intracellular defect responsible for the lessened capacity to transfer diodrast at high plasma levels, apparently paralleled, in a general way, the clinical and hematologic improvement. In contrast, the emergency treatment of shock results in a return of the cardiac output and blood pressure to normal before any change in renal hemodynamics occurs.<sup>7</sup> This lag between systemic and renal circulatory responses was not demonstrable in most instances of chronic anemia, possibly because improvement was slower. Where the anemia was rapidly corrected, as in H. C., by multiple transfusions of blood, marked renal vasoconstriction and depressed diodrast  $T_m$  continued to be apparent several days after the return of the hematocrit to normal.

It is interesting that a somewhat similar pattern of renal functional change has been described in diffuse glomerulonephritis.<sup>18</sup> The glomerular filtration rate is reduced in this disorder presumably by glomerular damage and destruction. Since filtration decreases more than the renal blood flow the filtration fraction falls. Unlike anemia, there is little evidence that renal vasoconstriction is important. Indeed, hyperemia as a result of vasodilation may be a prominent finding.<sup>18, 19</sup> It is probable that anemia may intensify this pattern of renal functional change, but it should be emphasized that anemia is not concerned with the initiation or perpetuation of the typical alterations in clearance values during the course of diffuse glomerulonephritis.

The findings of this study may throw light upon certain manifestations of chronic anemia. Edema occurs in a large number of cases on some basis other than decreased plasma oncotic pressure or increased venous pressure.<sup>20</sup> Salt and water are retained and the withdrawal of salt from the diet is frequently followed by loss of body weight and reduction of edema. In view of the consistent change in renal function

observed in the present study, it may be suggested that edema is secondary to renal retention of water and salt, possibly attributable to a glomerulotubular imbalance indicated in the reduction of the filtration rate/glucose Tm ratio. The reduction of the urea clearance and the development of azotemia may be attributed to a reduction of glomerular filtration rate, which may be quite severe (M. C., L. M., L. T., table 1).

The renal functional changes, in general, are nonspecifically related to chronic anemia. Patients with pernicious anemia showed a less marked deviation from normal, on the average, but since this group was made up largely of females, the possibility of a sex difference cannot be excluded. The circulatory adjustments are made independently of the cause of anemia and it is not surprising that the renal circulatory phenomena should follow suit.

Although hypertension may be produced experimentally by constricting the renal arteries and reducing the flow of blood through the kidneys, hypertension does not usually develop in the course of chronic anemia, even when renal ischemia is marked. There is no reason to believe that the circulatory adjustments of anemia prevent elevations of the blood pressure, since hypertensive emotional responses are easily evoked and the blood pressure may rise as the result of some other disease process, such as glomerulonephritis, in spite of severe anemia. Pre-existing hypertension persists, as in L. M., with very little change. These facts are opposed to the view that renal ischemia is an important causal factor in human hypertension.

#### SUMMARY

1. Renal function has been studied quantitatively in 15 patients with chronic anemia, 8 of whom were proved to have pernicious anemia. In 7 the anemia was secondary to chronic blood loss, iron deficiency, paroxysmal nocturnal hemoglobinuria, and leukemia. The effective renal plasma flow and glomerular filtration rate were measured by clearance technics; and tubular function, by saturation methods (diodrast Tm and glucose Tm).

2. The effective renal plasma flow, the glomerular filtration rate, and the filtration fraction (percentage of plasma filtered at the glomerulus) were reduced slightly below the normal values in most subjects. The effective renal whole blood flow was always greatly reduced, by 46 per cent on the average in males and by 31.8 per cent in females.

3. Since arterial pressure was not significantly depressed it was concluded that renal vasoconstriction occurs in chronic anemia, possibly as a homeostatic device for the diversion of blood to tissues more sensitive to oxygen lack. The relatively small reduction of filtration fraction implies afferent and efferent arteriolar vasoconstriction with dominance by the afferent arterioles. These changes were shown to be reversible, a return to normal values paralleling the return of the blood picture to normal.

4. Diodrast Tm was reduced significantly in 9 of 10 patients while the values of glucose Tm were normal in 6 of 7 patients. The normal values for glucose Tm indicated continued operation of all glomeruli and implied the absence of shunting or of cessation of blood flow in any significant portion of the kidney. The fall in

diodrast Tm, which appeared to be reversible in 2 of 4 individuals, was interpreted as evidence of intracellular dysfunction rather than destruction or inactivation of nephrons.

We wish to express our gratitude to the Misses Elizabeth Nolan and Constance Qua for technical assistance.

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## EDITORIAL

THERE is much talk about the country regarding the prospects of founding a national society for the study of the blood. The great surge of interest in this field and the increasing numbers of workers who spend most of their time in its study have naturally led to a desire for a central forum in which the subjects under discussion would for the most part be hematologic in nature, and where mutual problems could be aired. Beginnings in this direction have already been made through the formation of local societies in New York, Chicago, and elsewhere. The New York group has already made some preliminary soundings in the direction of forming a national society, and we understand that these were favorably received.

At the recent very successful International Hematology and Rh Conference, which was held in Dallas, Texas, on November 15-16 and in Mexico City on November 17-23, 1946, there was much talk of an international society. Physicians in the Latin-American countries in general express an avid interest in the possibility of forming an international American society to include both North and South America.

There can be but little quarrel with all these sentiments since they stem from the natural desire of men with mutual interests to convene. There may be some who look askance at the high degree of specialization which medicine has achieved and which has led to national societies for allergy, endocrinology, gastroenterology, neurology, and the like. Some thought must be given to this attitude, since by and large the allergist, the endocrinologist, the gastroenterologist, and the hematologist are better specialists in their respective fields if they are first of all good internists. However, any attempt to impede the inevitable growth of specialization seems futile on the face of it, since the complete encompassment of even a single specialty such as hematology with its many diverse facets has become well nigh impossible.

A society which is national or American (in the broad sense) seems to be definitely in the cards. The present journal, which has already achieved a world-wide circulation of about 2500 subscribers, may be said to be a start in this direction. The New York group has made tentative plans for an organizational meeting in conjunction with the centennial meeting of the American Medical Association at Atlantic City in June. With this plan we are fully in accord. It seems probable that before another year goes by an international society for the study of blood will have been organized. In this effort the journal BLOOD will extend all its cooperation and facilities. It would be of interest to receive the reactions and suggestions of interested readers.

WILLIAM DAMESHEK, M.D.

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# NEWS AND VIEWS

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## THE INTERNATIONAL HEMATOLOGY AND RH CONFERENCE, NOVEMBER 1946

Perhaps the first International Congress devoted solely to hematologic subjects to be held in the Western Hemisphere took place in Dallas, Texas, and Mexico City in November 1946. It began in Dallas on November 15-16 in affiliation with the second Mexican Congress of Blood Transfusion and then transferred its deliberations to Mexico City, where sessions were held from November 17 to November 23. Thus, the meeting was truly international as to both the participating sections and the meeting places.

The idea for such a meeting developed in December 1945 at a conference between Dr. Eduardo Uribe Guerola of the Juarez Hospital, Mexico City, and Dr. Joseph M. Hill of the Baylor Hospital, Dallas, Texas. Support for the proposal was given by the Mexican Government through the Department of Health headed by Dr. Gustavo Baz and by the Trustees of the Baylor Hospital. Additional financial support in Dallas was offered by various benefactors who had previously shown an interest in Baylor Hospital and its Blood Bank. It was originally planned to limit the program of the meeting to problems of the Rh factor. However, as interest in the proposed meeting rapidly mounted, and as a more general type of program seemed desirable, the final title of the meeting was changed to "International Hematology and Rh Conference."

Chairman of the Conference at Dallas was Dr. Joseph M. Hill, Pathologist at the Baylor Hospital; Secretary, Dr. Sol Haberman of the same hospital. Dr. Eduardo Uribe Guerola was Chairman of the Second Mexican Transfusion Congress and Dr. Alfonso Veléz Orozco, Secretary. There was a large attendance of physicians at the Dallas meeting, representing 21 states and the District of Columbia. In addition several physicians from Canada, Mexico, China, and England were present. Interest in the program which follows was intense:

Philip Levine, New Jersey:

Ernest Witelsky, Buffalo:

Ignacio González-Guzmán, Mexico  
City:

Robert R. Race, London, England:

Joseph M. Hill and Sol Haberman,  
Dallas:

"A Brief Survey of the Rh Factor"

"Interrelationship between the Rh  
System and the A-B System"

"The Nuclear Structure of the Blood  
Cells"

"The Rh Genotypes and Fisher's  
Theory"

"Rh Hemolytic Immune Globulins:  
Evidence for a possible Third Order  
of Antibodies Incapable of Agglu-  
tination or Blocking"

William Dameshek, Boston:	"Hemolytic Mechanisms"
Louis K. Diamond, Boston:	"Physiochemical and Immunological Character of the Rh antibodies"
Israel Davidsohn, Chicago:	"Rh Antibodies"
Mario Salazar Mallen, Mexico City:	"The Frequency of the Rh Factor in Different Groups of the Mexican Population"
Bruce Chown, Winnipeg, Manitoba:	"Variation in the Outcome of the Pregnancies in Which Erythroblastosis Occurs"

The Conference transferred to Mexico City from Dallas by plane at 4:30 A.M., Sunday, November 17, arriving in Mexico City about noon. Formal opening of the Second Mexican Blood Transfusion Congress took place in the Palace of Fine Arts, where addresses of welcome were delivered by, among others, Dr. Salvador Zubirán, Rector of the National University of Mexico and Director of the Hospital for Nutrition, and Dr. Gustavo Baz, Secretary of the Ministry of Public Health. A diplomatic reception was held on the evening of November 21 at the Ministry of Foreign Affairs, the host, a physician himself, being Dr. Francisco Castillo Najera, Minister of Foreign Affairs. Visits were made to the Hospital de Jesús, the oldest hospital in the Western Hemisphere, in continuous operation since 1554, to the Military Hospital, and to Xochimilco and the Pyramids.

Sessions were held daily, both the Spanish and English languages being used. The program was as follows:

J. M. Hill and Sol Haberman:	"Production and Use of Anti-Rh Serum"
Alfonso Veléz Orozco:	"The A and B Factors as Possible Causes of Erythroblastosis"
Sol Haberman and J. M. Hill:	"Demonstrations of Technics of Rh Testing"
R. R. Race:	"Subgroups of the Rh Factor. Demonstration of the Genetics of Rh and Other New Blood Groups"
A. V. Orozco and Rolando Medina Aguilar:	"Percentages of the Rh Subgroups in Mexico"
Philip Levine:	"The Individuality of Human and Animal Blood"
	"The Importance of the Rh Factor and Historical Development"
R. M. Aguilar:	"Use of A.C.D. Solution in Blood Banking"
I. González-Guzman:	"The Histopathology of Erythroblastosis"
Harry Wallerstein:	"The Treatment of Erythroblastosis by Complete Exchange Transfusion" (motion picture)

The discussions in Mexico City, although often lengthy, were lively and stimulating. At the concluding session\* on the morning of November 23, Dr. J. M. Hill, Chairman of the International Hematology and Rh Conference, and Dr. Eduardo Uribe Guerola, President of the Mexican Transfusion Congress, presided as co-chairmen. Discussions were held concerning the following problems:

(1) Organization of an international blood society with particular reference to experimental and immunohematology.

(2) Nomenclature of blood antigens and related antibodies. (3) Technics to be recommended for routine testing for these antigens and antibodies. (4) Availability of sufficient Rh serum for routine typing in transfusions and pregnancies.

After a very active and complete discussion, recommendations and actions were taken by this joint conference. Two committees were appointed with authorization to function as indicated. The first committee was charged with the responsibility for taking the necessary steps to form an international organization to carry on the work begun at this meeting. This committee was also instructed to undertake the standardization of blood typing serums and to promote the production of Rh serums from human sources as a cooperative project. This committee consists of Dr. J. M. Hill, Dallas, Texas, Chairman; Dr. William Dameshek, Boston, Mass.; Dr. Louis K. Diamond, Boston, Mass.; Dr. Luis Gutierrez Villegas, Mexico, D.F.; Dr. Philip Levine, Raritan, New Jersey; Dr. E. A. Mourant, London, England; Dr. W. S. Stansbury, Toronto, Canada; Dr. Eduardo Uribe Guerola, Mexico, D.F.; and Dr. Ernest Witebsky, Buffalo, New York.

The second committee was appointed to study and recommend nomenclatures for blood antigens and technics to be used for routine blood typing and antibody investigations. This committee consists of Dr. Philip Levine, Raritan, New Jersey, Chairman; Dr. Bruce Chown, Winnipeg, Canada; Dr. Israel Davidsohn, Chicago, Illinois; Dr. Sol Haberman, Dallas, Texas; Dr. J. M. Hill, Dallas, Texas; Dr. R. R. Race, London, England; and Dr. Eduardo Uribe Guerola, Mexico, D.F.

In the discussions the advantages of the Chown capillary and Diamond slide technics for routine Rh typing were emphasized. For detection of antibodies the Coombs ("developing") test and the Diamond albumin test received favorable comment. The compatibility test of Witebsky was also highly recommended. Review of these and other technics was referred to the committee.

It was of interest that the Fisher-Race theory of inheritance and the CDE nomenclature suggested by these workers were felt to be most suitable for serologic and genetic study of Rh and Hr subgroups and were recommended for acceptance temporarily until the problem could be more thoroughly reviewed by the committee. The members of the joint meeting also decided to retain, at least for the immediate future, the term "erythroblastosis foetalis" for cases resulting from isoimmunization of mothers by the Rh, Hr, and other blood antigens. The use of the terms "Rh positive" and "Rh negative" along with "homozygous" and "heterozygous" was retained for clinical use. The use of the terms "X-protein" and "conglutination" was rejected on the basis of lack of evidence.

\* The report of this session was furnished by Dr. Sol Haberman.

Routine Rh typing for all transfusions and pregnancies was strongly recommended. However, it was suggested that when adequate quantities of anti-Rh serum were not available, only women should be routinely Rh tested. Testing of women only was also recommended when the population or race concerned was known to have a very small percentage of Rh negatives. It was agreed that anti-Rh serum for routine testing should contain the anti-D antibody (85 per cent) or anti-D+C (87 per cent).

The meetings in both Dallas and Mexico City proved highly successful. Their international character was of help in fostering mutual good will among those participating. It was agreed by all that further conferences of this sort, dealing perhaps with other subjects and of a more general character, were worth while. It seemed eminently desirable to initiate by every means possible the formation of an International Society for Hematology. It was also agreed to attempt the publication of the various papers at both sections of the Congress in a special issue of *Blood*.

inverted with a deep S wave. S-T is depressed in leads 1 and 2 and T is inverted in leads 2 and 3. The T inversions in the chest leads may be normal for a patient of the

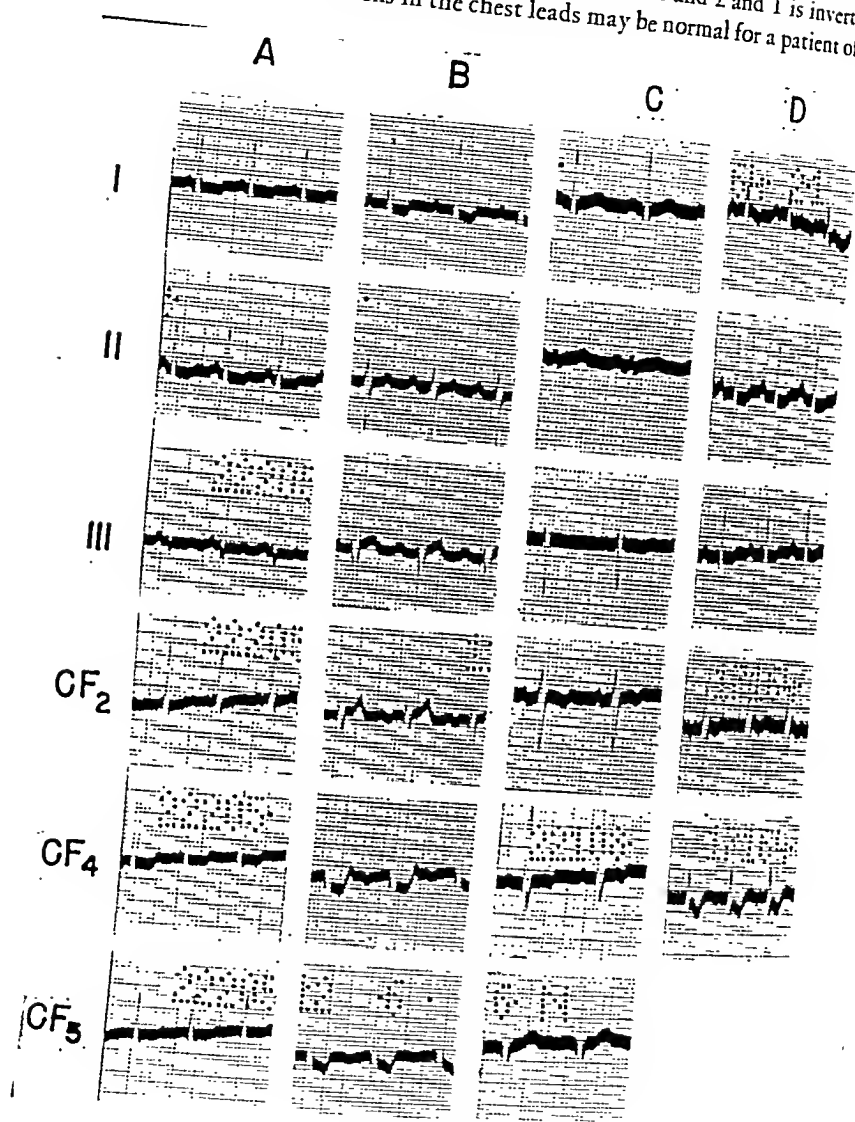


FIG. 1

age (6 years). The interpretation is: sinus tachycardia, right axis shift, possibly right heart strain, a definitely abnormal record.

Figure 2B, taken ten days before death, shows a sinus tachycardia. A small Q wave is present in lead 3. The T waves are small in the limb leads. The inversion of the T waves in leads CF<sub>2</sub> and CF<sub>4</sub> may be normal for a child (3 years old). The interpretation is: sinus tachycardia, a borderline record.

Figure 2C, taken two months before death, shows depression of the S-T segments in all of the limb leads.  $T_2$  is diphasic and  $T_3$  inverted. The T wave changes in  $CF_2$

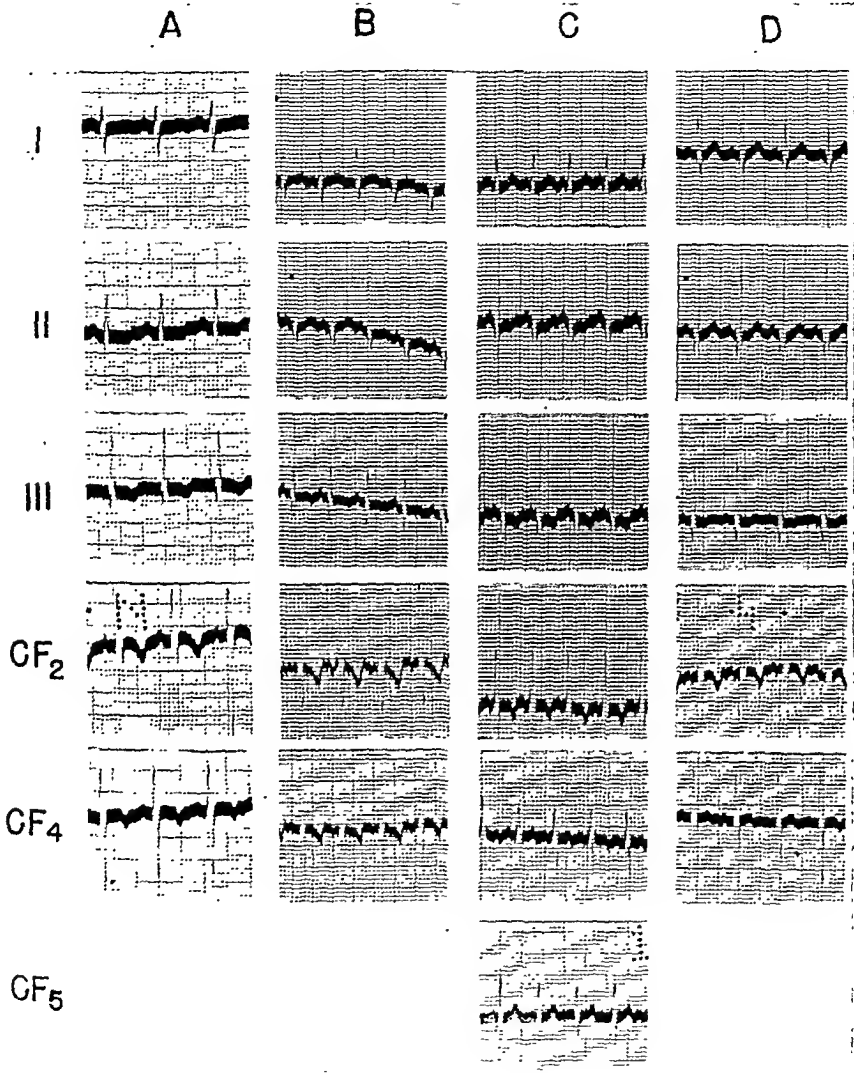


FIG. 2

and  $CF_4$  could be normal for the age of this patient ( $5\frac{1}{2}$  years). The interpretation is: sinus tachycardia, right axis shift, a definitely abnormal record.

Figure 2D, taken five weeks prior to death, shows a sinus tachycardia. A small Q wave is present in leads 2 and 3 and S-T is depressed in these leads. The T wave changes in the chest leads could be normal for a child of this age ( $4\frac{1}{2}$  years). The diagnosis is: sinus tachycardia, a borderline record.